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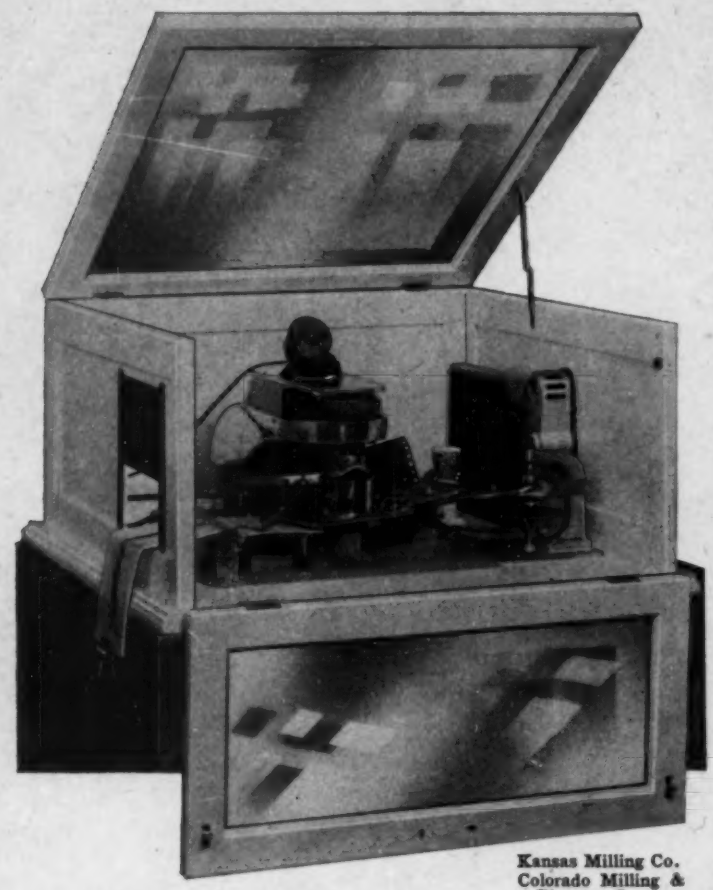

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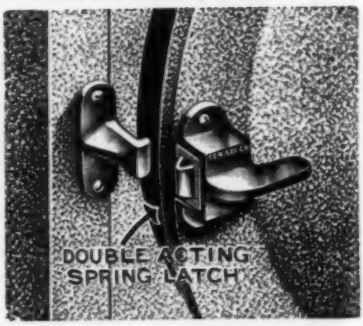

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VOL. XXIII JANUARY, 1946 No. 1

METHODS FOR DETERMINATION OF ALPHA-AMYLASE. II. LIQUEFYING AND DEXTRINIZING ACTIVITIES OF ALPHA-AMYLASES FROM DIFFERENT SOURCES

SUTTON REDFERN and QUICK LANDIS [1]

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(Read at the Annual Meeting, May 1944; received for publication July 27, 1945)

Three general methods have been used in the study of amylases, viz., saccharification, dextrinization, and liquefaction. It has been amply demonstrated that saccharification methods measure a summation of the effects of alpha- and beta-amylase. Dextrinization methods based on the original method of Wohlgemuth (1908) also measure the combined effect of alpha- and beta-amylase as shown by Sandstedt, Kneen, and Blish (1939) and Hanes and Cattle (1938). No methods are available for determining the amount of beta-amylase without correction for any alpha-amylase that may be present concomitantly. There are, however, two methods at present available for specifically determining alpha-amylase: the measurement of the dextrinization time in the presence of an excess of beta-amylase and the liquefaction procedures of Jozsa and Johnston (1935) and Blom and Bak (1938).

Hollenbeck and Blish (1941) presented data which showed satisfactory parallelism of the effects of temperature changes on liquefying and dextrinizing activities for three types of alpha-amylases: malt, bacterial, and fungal. Dickson (1943) also found from a collaborative study on a series of malts that there was a high correlation between the liquefying and alpha-dextrinizing methods, indicating that these methods were measuring the same thing. Dickson's work was done on only one type of alpha-amylase, viz., malt. All of Hollenbeck and Blish's experiments except one were done by inactivation studies of individual amylases. Thus, if 50% of the enzyme as measured by the liquefying method was inactivated, then approximately 50% was inactivated as measured by the dextrinizing method.

[1] Deceased.

These authors went one step further, however, and stated that when extracts of the three enzymes were adjusted to an equal basis in terms of alpha-dextrinizing power, their liquefying activities also were found to be substantially the same. Only one experiment to test this was given and the outflow times of the starch pastes which should have been equal did not agree too well.

The present paper resulted from attempts to intercompare alpha-amylases from different sources by the two methods. Certain discrepancies were noted which it was felt required further investigation before the methods could be properly standardized according to the principles developed by Landis (1945).

Types of Enzymes Used

Three types of amylases were selected for study, those of barley malt, bacteria, and mold. The different enzyme preparations studied and their sources are described in Table I.

TABLE I

DESCRIPTION OF ENZYMES

Enzyme	Source	Commercial name
Malt	Barley malt	Dry distillers malt
Malt syrup A	Barley malt	T.P. Diastafor
Malt syrup B	Barley malt	Heavy Diastafor
Malt syrup C	Barley malt	Special Diamalt
Malt syrup D	Barley malt	Regular Diamalt
Takadiastase A	Mold	Takadiastase
Takadiastase B	Mold	Takadiastase
Fungal A	Mold	Clarase 700
Fungal B	Mold	Rhozyme
Fungal C	Mold	Maltase Prepn. No. 20
Bacterial A	Bacteria	Laboratory Prepn.
Bacterial B	Bacteria	Rapidase, conc.

Methods

Liquefying Activity. The viscometric method developed by Landis (1943) was used for the determination of the liquefying activity. This method is considerably more convenient to use than the regular Jozsa and Johnston method and permits one to run many more samples in one day. The details of the method will be given in the following paper of this series. In brief, the method consists of determining the percentage liquefaction produced by the enzyme acting on a special starch substrate. A constant time of one hour, a pH of 5.2, and a temperature of 30°C was used for all the dilution curve work.

The enzyme solutions were prepared with 0.025 molar calcium chloride solution.

Dextrinizing Activity. Three methods were used. The regular method as described by Sandstedt, Kneen, and Blish (1939) was used to determine the relative alpha-dextrinizing (SKB) activities of the different enzymes, and the same method without addition of excess beta-amylase to determine the relative Wohlgemuth activities.

In order to be able to study the kinetics of the dextrinization reaction, a colorimetric procedure devised by Landis (1943) was used. The substrate was prepared according to the directions of Sandstedt, Kneen, and Blish. An inorganic color standard which was found to compare closely with the iodine-dextrin solution of these authors was used for color comparisons. It consisted of 20 g $CoCl_2.6H_2O$, 3 g $K_2Cr_2O_7$, and 10 ml of 0.1 *N* HCl made up to 100 ml with distilled water. A Klett-Duboscq colorimeter was used. The proportions of enzyme infusion to substrate solution were as specified in the method of Sandstedt *et al.* At appropriate time intervals, 2 ml of the reacting mixture were added to 10 ml of iodine solution "B" and color comparisons were made. A Wratten "A" red filter was placed in the eyepiece of the colorimeter in order to improve the ease of matching without having to take into account the color changes taking place in addition to intensity changes. The standard solution was used at a depth of 20 mm. The unknown colorimeter readings expressed in millimeters were used directly as a measure of the amount of substrate changed.

The enzyme solutions for the dextrinizing method were also prepared with 0.025 molar calcium chloride solution.

Intercomparison of the Liquefying and Dextrinizing Strengths of the Different Amylase Preparations

In order to put the liquefying determinations on a basis which does not depend on calibration or initial rates and is independent of any enzyme units, the method of "equivalence by superposition" (Landis, 1945) is used. By this method, the amount of each enzyme preparation required to produce a given amount of conversion in a given time is determined. However, in order to make it practical, different concentrations of the enzyme were used and the amount of conversion in constant time is determined. By interpolation, the amount of enzyme to give any intermediate degree of conversion is easily determined and the results may be checked at several points by this technique.

The dilution curves determined for each of the three types of

alpha-amylases are shown in Figure 1. For the malt and bacterial amylases the curves are essentially parallel straight lines over the dilution range studied. The takadiastase dilution curve is markedly different. The ratio of the enzyme strengths of these three preparations may be calculated, from the weight of each enzyme required, to give any degree of conversion as read from the curve. The ratio may also be calculated graphically by measuring the distance between the curves since the sample weights are plotted on a logarithmic scale.

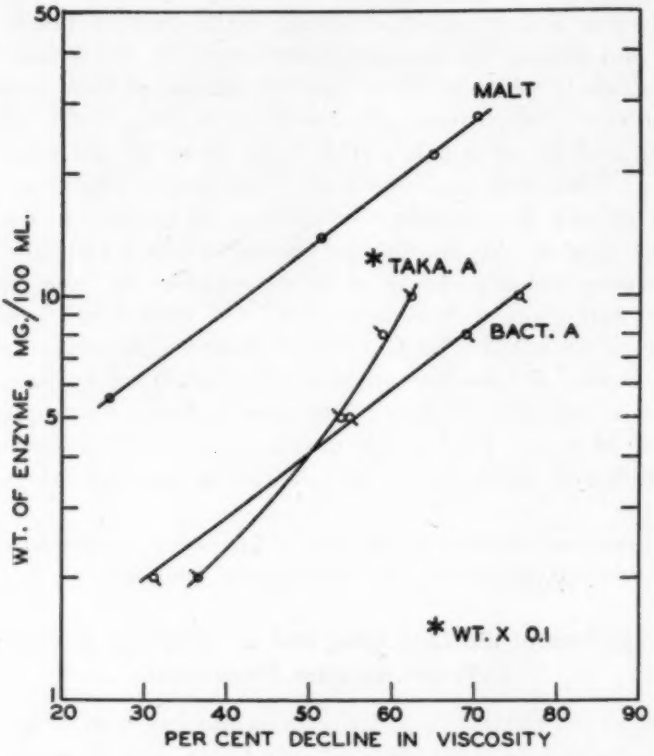

Fig. 1. Liquefaction-dilution curves for different alpha-amylases.

This method of plotting makes it possible to determine by mere inspection whether the enzymes can be compared at different degrees of conversion since the curves will be parallel if this is true. Since the enzyme strengths are inversely proportional to the equivalent weights, it is easy to calculate the strength ratios, assuming the malt to have a value of unity at any given percentage decline in viscosity. These values are shown in Table II.

Table II shows that the malt and bacterial enzymes may be compared at any degree of conversion since the dilution curves are

TABLE II

COMPARISON OF RELATIVE STRENGTHS FOR DIFFERENT ENZYMES

Enzyme	Ratio of strengths						
	Percentage decline in viscosity						SKB[1]
	40	45	50	55	60	70	
Malt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bacterial A	3.24	3.24	3.24	3.22	3.22	3.22	2.28
Takadiastase A	39.2	36.3	33.0	28.4	23.1	—	29.9

[1] Refers to the Sandstedt, Kneen, and Blish method.

parallel. The takadiastase varies and when compared to malt its activity varies almost twofold depending upon the degree of conversion selected for comparison.

The relative strengths of these three enzymes as determined by the alpha-dextrinizing method are also shown in Table II. It is to be noted that the three preparations definitely show different relative strengths when compared by the two methods. This is contradictory to Hollenbeck and Blish's work.

It might be questioned that these results were peculiar to the three samples of alpha-amylases used. That this is not so is shown in Figure 2, where the dilution curves of a malt syrup, another bacterial preparation, and three different fungal amylases are compared. One of the fungal enzymes, sample C, was developed for a high maltase activity, but this did not significantly alter the curve. Again we find that the bacterial and malt amylase dilution curves are essentially parallel, while the fungal enzyme curves are different, although they are all roughly parallel to one another.

It must be noted that one set of curves cannot be compared exactly with another set. Each set represents determinations made on one batch of substrate in order to eliminate the question of unavoidable daily differences caused by the inability to duplicate the initial viscosity of the substrate accurately on different days.

Since the three types of alpha-amylases show different strength ratios by the two methods, it is difficult to understand how Hollenbeck and Blish found equivalent outflow times for a starch paste liquefied by equivalent alpha-dextrinizing concentrations for a constant time. Nevertheless, a further check of this point was made. A careful analysis of samples of the three enzymes gave relative alpha-dextrinizing and liquefying ratios as shown in Table III.

Using weights of each enzyme inversely proportional to the alpha-dextrinizing ratios, liquefying rate curves were run, as shown in

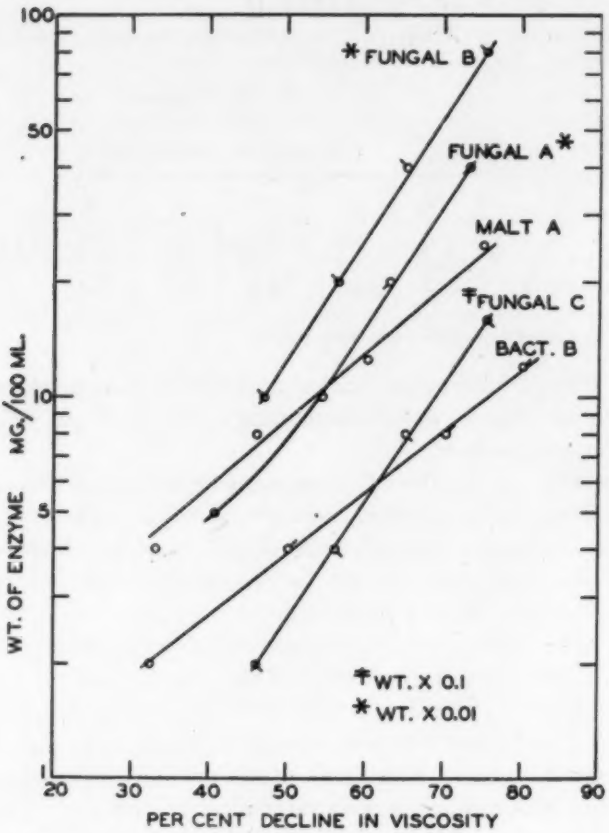

Fig. 2. Liquefaction-dilution curves for different alpha-amylases.

TABLE III

RELATIVE ENZYME STRENGTHS USED FOR RATE CURVES

Enzyme	Relative strengths by	
	Liquefaction	Alpha-dextrinization
Malt syrup A	1.0	1.0
Bacterial A	2.03	1.28
Takadiastase A	23.6[1]	19.4

[1] Equivalent initial rate.

Figure 3. The curves are not superimposable, which they should be if the two types of activities are equivalent. The bacterial amylase is evidently more active in the liquefying method than in the dextrinizing method. The takadiastase is quite different. It produces a rapid initial decrease in viscosity, then the rate falls off. The experimental results seem to indicate that the results of Hollenbeck and Blish were

perhaps fortuitous. Calculations from their data show that the final outflow times are equivalent to a viscosity decline of approximately 92%. At this point the viscosity change is rather slow and any real differences may have been masked.

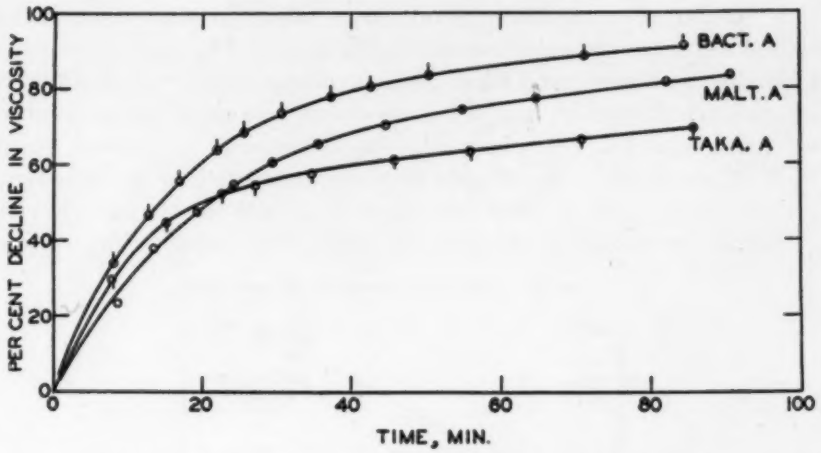

Fig. 3. Liquefaction rate curves for equivalent alpha-dextrinizing quantities of different alpha-amylases (malt A, 25.0 mg; bacterial A, 19.5 mg; takadiastase A, 1.29 mg; all per 100 ml).

Liquefying rate curves for equivalent liquefying quantities of the enzymes were also studied, with the same quantity of malt syrup used above as a basis. In this case an amount of takadiastase was chosen to give an initial rate approximately equal to the other two. Figure 4

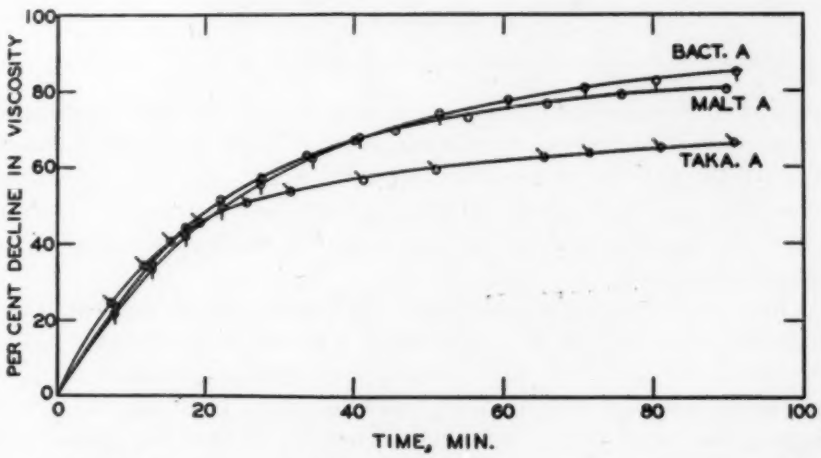

Fig. 4. Liquefaction rate curves for equivalent liquefying quantities of different alpha-amylases (malt A, 25.0 mg; bacterial A, 12.3 mg; takadiastase A, 1.06 mg; all per 100 ml).

shows that the malt and bacterial amylases give nearly equivalent rate curves, but again the takadiastase exhibits an entirely different kinetic picture. The rate very quickly falls off and the enzyme action slows down markedly so that the amount of work done in the longer times is much less than would be calculated from the initial rate.

A similar dilution curve study was made for the dextrinizing method using Landis' colorimetric modification. The ordinary method of Sandstedt, Kneen, and Blish gives a unique answer when different enzymes are compared, but yet if they were compared at some other end-point than the one chosen by these authors, a different result might be obtained, as has already been demonstrated for the liquefying method. The same general procedure was used for the colorimetric method as for the liquefying method. The colors produced by varying

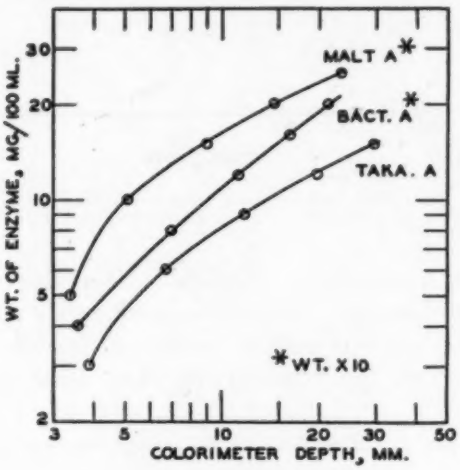

Fig. 5. Colorimetric alpha-dextrinizing dilution curves for different alpha-amylases.

quantities of enzyme in constant time were determined and the dilution curve plotted on log-log coordinate paper. These scales were chosen merely to give approximately straight lines. Exactly the same reasoning as in the liquefying method was used. At equal colors in constant time the enzymic strengths are in the inverse ratio of the weights required.

Figure 5 shows the colorimetric dilution curves for the three enzymes. Again, we find differences between the three types of enzymes. In this case, however, the malt and fungal enzyme curves are nearly parallel and the bacterial one differs from the other two. It may be noted that a colorimeter reading of 20 mm corresponds approximately to the regular dextrin-iodine end-point color. Table IV gives the relative strengths obtained from Figure 5.

TABLE IV

RELATIVE STRENGTHS OF DIFFERENT ENZYMES AT DIFFERENT COLOR END-POINTS

Enzyme	Relative strengths							
	Colorimeter readings, mm							
	4	6	8	10	15	20	25	Avg.
Malt syrup A	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bacterial A	2.0[1]	1.72	1.61	1.52	1.37	1.28	1.20	1.45
Takadiastase A	27.0[1]	23.0	21.1	20.1	19.0	18.5	18.0	19.9

[1] Not included in average.

The ratios obtained at a reading of 20 are, within the experimental error, the same as the ones already obtained by the regular dextrinizing method. At other colors the fungal enzyme gives approximately the same relative strengths when compared with the malt enzyme, but the bacterial enzyme gives varying results. Therefore, the dextrinizing method also gives results which depend on the end-point.

Intracomparison of the Liquefying and Dextrinizing Strength of Different Amylase Preparations

Kneen, Sandstedt, and Hollenbeck (1943) have noted that "beta-amylase prepared from barley or wheat is applicable in the preparation of a substrate for the determination of *cereal* alpha-amylase activity, but the advisability of its use for the determination of the activity of other 'alpha-amylases,' such as those of animal or microbial origin, has not been established and is not recommended." Nevertheless, from preliminary work, it seemed that either a dextrinizing method or a liquefying method could be used for intracomparisons of the different alpha-amylases, but not intercomparisons.

Experiments to check this were made as follows. Dilution curves for four types of malt syrups were made and from these the relative activities are determined. The dilution curves are shown in Figure 6 and the ratios of their strengths are shown in Table V. The average ratios are compared with the values determined by the alpha-dextrinizing method in Table VI.

The agreement is very good when it is considered that the error of each method is approximately $\pm$ 5%. To further check the agreement, rate curves for equivalent alpha-dextrinizing quantities of enzyme were run. The agreement is so good that it is too difficult to show on a graph because the points all run together. The maximum deviation between the curves is 1.2% decline in viscosity, which is excellent. It may be concluded that a series of malts may be intracompared by either the alpha-dextrinizing or the liquefying method.

TABLE V

COMPARISON OF LIQUEFYING METHOD FOR MALT SYRUPS

Malt syrup	Relative strengths						
	Percentage decline in viscosity						
	35	40	50	60	70	75	Avg.
A	1.0	1.0	1.0	1.0	1.0	1.0	1.0
B	1.41	1.42	1.44	1.46	1.48	1.48	1.45
C	0.324	0.322	0.320	0.315	0.310	0.308	0.317
D	0.138	0.138	0.140	0.142	0.135	0.128	0.137

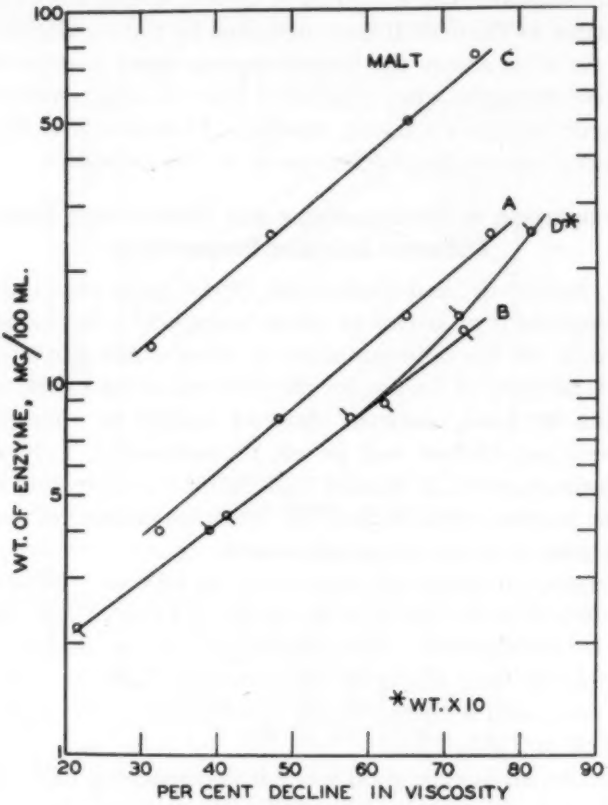

Fig. 6. Liquefaction-dilution curves for malt syrups.

The same study was made for bacterial amylases. In order to prepare samples of varying strength, some of the original material was boiled to kill the enzyme, and this boiled enzyme solution used to dilute the original material. By this means each solution varied

TABLE VI

COMPARISON OF RELATIVE STRENGTHS OF MALT SYRUPS BY LIQUEFYING AND ALPHA-DEXTRINIZING METHODS

Malt syrup	Relative strengths		
	Liquefying	SKB	Δ%[1]
A	1.0	1.0	—
B	1.45	1.42	− 2.1
C	0.317	0.271	−14.5
D	0.137	0.124	− 9.5

[1] Percentage deviation of SKB value from liquefying value.

essentially only in its enzyme content. At least all thermostable substances are retained. Three different preparations were compared by liquefying dilution curves, dextrinizing and alpha-dextrinizing activities. The results of the liquefying dilution curves are given in Figure 7 which shows their parallelism. The comparison between the methods is given in Table VII. The agreement between all three

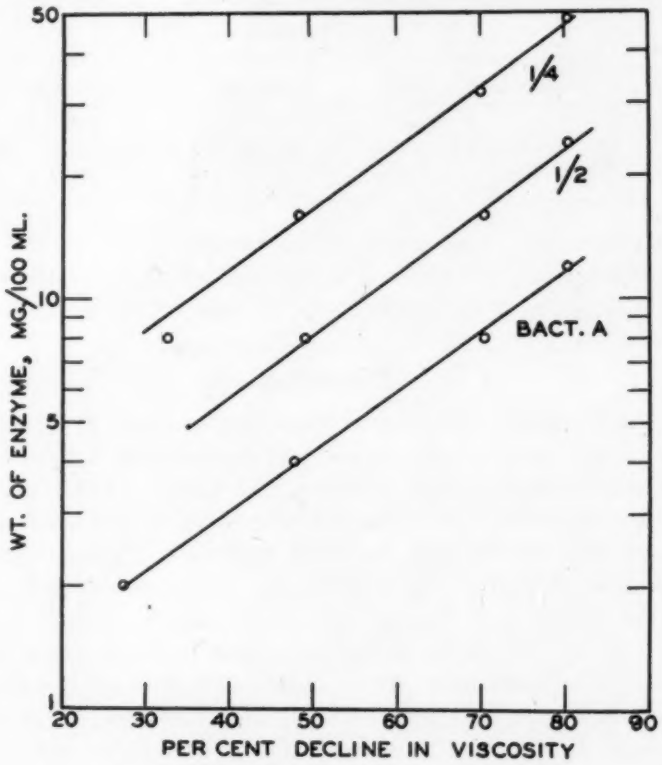

Fig. 7. Liquefaction-dilution curves for bacterial amylases.

TABLE VII

COMPARISON OF RELATIVE STRENGTHS OF BACTERIAL AMYLASES BY LIQUEFYING AND DEXTRINIZING METHODS

Bacterial amylase	Relative strengths				
	Liquefying	Wohlgemuth	SKB	Δ% Wohl.	Δ% SKB
A	1.0	1.0	1.0	—	—
A−½	0.517	0.488	0.500	−5.6	−3.3
A−¾	0.255	0.244	0.244	−4.3	−4.3

methods is excellent and it may be concluded that bacterial amylases may be intracompared by all three methods.

The same study was made of different fungal enzyme preparations. Using the values obtained from Figure 2, the relative strengths are compared in Table VIII. The agreement between the methods is

TABLE VIII

COMPARISON OF RELATIVE STRENGTHS OF FUNGAL ENZYMES BY LIQUEFYING AND DEXTRINIZING METHODS

Fungal amylase	Relative strengths				
	Liquefying	SKB	Wohlgemuth	Δ% SKB	Δ% Wohlgemuth
A	1.0	1.0	1.0	—	—
B	0.590	0.574	0.568	−2.7	− 3.7
C	0.287	0.272	0.324	−7.7	+12.9

quite satisfactory. The liquefaction dilution curves are not as parallel as shown for the malts or bacterials, but the deviations are not great. Any of the three methods can be used to intracompare fungal enzymes.

Discussion

The cause of the differences between the three types of alpha-amylases is quantitative since qualitatively all cause the same general type of starch degradation. Hanes and Cattle (1938) also found quantitative differences between different types of amylases, although their work did not include bacterial amylase. Pigman (1943) has found similar differences for invertases from different sources. He found that the ratio of sucrase to inulase activity varied from 5 to 4,000. The ratio should have been constant, if all the invertases were identical. The invertases are a class of enzymes. Similarly, the amylases are a class of enzymes which differ according to their source.

It might be thought that concomitant materials may cause the differences. Some dilution curves were run, for example, on partially

inactivated takadiastase, where the inactivation was done by a short heating to 50°C. The dilution curves were all parallel, although in one case the sample contained four times as much of the concomitant materials as the original sample. Another test was made by adding some boiled malt, boiled bacterial amylase, and soybean extract to a solution of takadiastase. The percent decline in viscosity was, within the experimental error, the same for all the solutions as shown in Table IX.

TABLE IX

EFFECT OF VARIOUS ADDITIONS ON THE LIQUEFYING ACTIVITY OF TAKADIASTASE

Enzyme solution	Decline in viscosity in one hour
	%
Takadiastase alone, 1.06 mg	62.9
Takadiastase + extract from 1 g soybean	63.1
Takadiastase + 250 mg boiled malt	63.0
Takadiastase + 125 mg boiled bacterial enzyme	62.5

Another cause of the differences may be union of part of the enzyme with the reaction products to form an inactive compound, or, as suggested by Hanes and Cattle, the different enzymes may have varying affinities for the lower dextrins which are produced.

A different pH is used in each of the two methods, viz., 4.7 for the dextrinizing method and 5.2 for the liquefying method. Since it is usually accepted that bacterial amylase has a higher pH optimum, it was thought that the difference in pH levels may have accentuated the differences, especially for the bacterial amylase. Therefore, the three enzymes were tested by the liquefying method at pH values from 4 to 8. Little difference in activity was found between the values of 4.75 and 6.0. It is concluded that the difference in pH levels does not significantly contribute to the difference between the methods reported herein.

One of the objects of this work was to devise a satisfactory method for intercomparison of the three types of enzymes. It does not seem possible to intercompare malt and bacterial alpha-amylases by the two different methods. Since the liquefying method gives the highest value of the relative strengths, it is perhaps preferable to use this value as representing the maximum activity that can be obtained from a given weight of enzyme preparation. A more extended study of the dilution curve of fungal amylase was made and the results are given in Table X. It was found that between the limits of 15 to 50% decline in viscosity the relative strength of the fungal amylase compared with barley malt is reasonably constant. This can be used as

TABLE X

RELATIVE STRENGTH OF TAKADIASTASE B COMPARED TO MALT SYRUP A

Decline in viscosity		Relative strength
%		
15		25.4
20		28.0
25		28.4
30		28.4
35		28.4
40		27.7
45		26.0
50	Avg. = 26.9	23.3
55		20.0
60		17.0
65		15.0
70		13.1
75		11.8
80		10.8

a means of securing some agreement with the malt and bacterial amylases.

If the strength is measured in this range, the value will be a maximum. In general, it is desirable to measure enzyme strengths on a basis of initial rates as nearly as possible. The liquefying method is superior in this respect to the dextrinizing method since very little change of the starch takes place during the liquefying period.

It is not to be expected that the maximum enzyme values, as determined in the laboratory under a very specialized set of conditions, will necessarily agree with the relative values determined under plant or practical conditions. The best that we can hope to do in the laboratory is to devise a measure of the number of enzyme molecules that are present. How this number of enzyme molecules will work under a different set of conditions has to be determined experimentally.

Summary and Conclusions

A comparison of the relative liquefying and dextrinizing activities of alpha-amylases from three different sources, malt, bacteria, and mold, was made.

It was found that the relative strengths determined by the liquefying method were independent of the degree of liquefaction for the malt and bacterial amylases. They were not independent for the fungal amylases. However, the relative strength of the fungal amylase was found to be a maximum in the range 15 to 50% decline in viscosity, and measurements in this region are proposed as a measure of the maximum strength obtainable from a fungal amylase.

Liquefaction rate curves for equivalent alpha-dextrinizing amounts of the three types of amylase were not superimposable, which they should have been, if the two methods are equivalent. It is not strictly possible to intercompare the different amylases by the two methods, although on the basis of initial liquefying rates there is reasonable agreement for the malt and fungal amylases. If measurements are restricted to one type of amylase, they may, however, be intracompared by either the liquefying or alpha-dextrinizing method. Bacterial and fungal amylases may also be intracompared by a simple Wohlgemuth iodine method.

The alpha-amylases of malt, bacteria, and mold are a class of enzymes which differ quantitatively in their action on starch. (It is self-evident that pancreatic amylase is also a member of this class, but no experimental work was reported because of the considerable difference in optimum pH.)

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SUPERCENTRIFUGATES FROM DOUGH

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During the mixing of bread dough much of the water remains free to dissolve flour solubles, as suggested by Newton and Gortner (1922), and later determined by Skovholt and Bailey (1935) and Vail and Bailey (1940). This aqueous solution doubtless forms a liquid phase in the dough structure. The object of this paper is to describe a method of separating an aqueous solution from dough and the determination of its composition and properties.

The solubles in dough have heretofore been studied only in dilute solutions made by extracting flour, or leaching doughs dispersed in water. In recent studies on pentosans by Baker, Parker, and Mize (1943) and on sulfhydryl compounds of flour by Baker, Parker, and Mize (1944), flour solubles in a more concentrated form were obtained from centrifuged batters. The action of a high speed centrifuge on these batters suggested that some of the liquid phase of dough could be obtained in an unaltered and undiluted condition by centrifuging the dough in a higher speed machine. A Sharples centrifuge operated at 40,000 rpm using 200 g of dough in a closed bowl gave the desired results. The work here reported concerns the liquid which was drained from the surface of the supercentrifuged dough, the remainder of which remains firmly packed on the walls of the bowl.

Working (1934) previously supercentrifuged batters to determine absorption, but used less severe centrifugation since the dough remaining in the machine was presumed to retain the optimum amount of water for baking bread.[1]

The liquid obtained by supercentrifuging dough was analyzed and its properties determined. Variations among flours and the changes resulting from dough operations in breadmaking or from special ingredients added to dough or to flour were determined. The relation of the liquid phase of dough to breadmaking was further studied by using the supercentrifugate as the liquid for the manufacture of bread.

Methods and Materials

Preparation of Doughs. Doughs were made from a variety of flours in a closed McDuffy bowl with a Hobart C 10 mixer in the presence of carbon dioxide. A speed of 65 rpm was maintained for

[1] The speed of the centrifuge at the time of operation was not stated by Working, but in a private communication Dr. C. O. Swanson advised that a speed of 10,000 rpm was used.

stated periods of time. An absorption of 73% was used for all flours corrected to 12% moisture. This amount of water was required to provide sufficient liquid from a single supercentrifugation so that all desired analytical data could be obtained. Many flours at normal absorption yield only small amounts of liquid. Also, this uniform absorption permits direct comparison of all data.

For all fermented doughs the following formula was used: 200 g of flour at 12% moisture basis, distilled water to 73% absorption, 4 g of salt, and 5 g of yeast. In nonfermented doughs the yeast was, of course, eliminated from the above formula.

Preparation and Analysis of Supercentrifugates. Immediately after mixing, 200 g of dough was supercentrifuged in the closed bowl of a Sharples supercentrifuge at a speed of 40,000 rpm for 20 minutes. The centrifuge was mounted in an insulated enclosure, the temperature of which was controlled so that the dough which left the mixer at a temperature of 29.4°C was held within ± 1°C during centrifugation. Full air pressure was applied intermittently to distribute the dough and obtain smooth operation, after which 30 pounds of air pressure was applied to operate the machine at full speed for 20 minutes.

During the operation part of the starch was forced to the bowl wall, gluten and starch formed an intermediate layer, and the free, very viscous liquor was crowded to the inner surface. Upon completion of the supercentrifugation the liquor was separated by gravity drainage for 2 minutes and weighed.

A gel test which, in part, was previously described by Baker, Parker, and Mize (1943) was performed on 3 ml of the supercentrifugate as follows: The liquor was forced from a pipette as a jet into 2 drops of 3% hydrogen peroxide contained in a 25 ml test tube. This procedure gave a uniform mixture. After an hour of contact, the rigidity of the gel was judged empirically, employing values from 0 to 8.

The liquor as originally separated could not be accurately sampled because of its high viscosity and the presence of small amounts of suspended material. Dilution with distilled water formed a precipitate. It was therefore diluted with 2 volumes of a 2.67% salt solution, this being the average concentration produced from the water and salt added to the dough. No precipitate was produced thereby. The traces of suspended matter were readily removed from the diluted solution by further centrifugation in covered cups. Weighed samples of about 5 g of the diluted solution were used to determine solids, pentosans, protein, and sodium chloride. All determinations were made in duplicate and results averaged. Solids were determined by evaporating a weighed sample of approximately 5 g to constant weight

on a steam bath. Soluble pentosans were determined as outlined in *Cereal Laboratory Methods* (4th ed., 1941, p. 68) by distilling once. Preliminary tests indicated that redistillation did not appreciably lower the values. Soluble protein was determined by the Kjeldahl method as specified in *Cereal Laboratory Methods* (4th ed., 1941, p. 34). To determine the salt content, the dried soluble extract was ashed in the presence of 5 g of a mixture of 6 parts potassium carbonate, 4 parts sodium carbonate, and 3 parts potassium nitrate. This ash was then extracted with nitric acid and the chloride content determined, as described in *Cereal Laboratory Methods* (4th ed., 1941, p. 129). This result was corrected for the salt added in the dilution water. All results were calculated to percentage by weight of the original supercentrifugate.

The percentage of sugar was calculated from the difference between soluble solids and the sum of the soluble pentosan, soluble protein, and sodium chloride.

The viscosity of the diluted liquor recorded in seconds was determined in an Ostwald-Fenske pipette (Cannon and Fenske, 1936) and characterized by the number 300 with a water effluent time of 5 seconds. All measurements were made at 30°C.

The sulfhydryl determinations were carried out upon 0.5 ml of the diluted supercentrifugate as described by Baker, Parker, and Mize (1944) and calculated as ppm of glutathione in the original supercentrifugate.

The relation of sodium chloride to water was calculated from the total sodium chloride and water found in the original supercentrifugate. This calculation is made by the division of the percentage of sodium chloride by the sum of the percentage of sodium chloride and percentage of water and the multiplication of this quotient by 100. The percentage of water is determined by subtracting the percentage of soluble solids from 100%.

The resistance to puncture of hand-washed glutens and of glutens purified by two dispersions, both being in 2.67% sodium chloride solutions, was determined as described by Baker, Mize, and Parker (1943).

Results from Different Flours

The supercentrifugates from a series of 25 flours were prepared and analyzed. The results are reported in Table I showing the average, maximum, and minimum values obtained from these analyses. The analytical results obtained are not reported for each flour because of their voluminous nature. A series of five flours is reported to show some of the variations found in individual flours. It is not the in-

TABLE I

COMPOSITION OF SUPERCENTRIFUGATES SEPARATED AT ONCE FROM DOUGHS MIXED 1½ MINUTES
(Averages from 25 flours of 1943 and 1944)

Flour	Flour protein 12% moisture	Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	-SH as G-SH	Force to puncture gluten	
												Crude	Pure
	%	g		sec	%	%	%	%	%	%	ppm	g	g
Average of 25 flours	12.2	23.8	6.5	30.8	13.1	6.61	1.55	2.61	2.33	2.62	180	10.2	28.2
Maximum	14.9	37.5	8.0	105.0	18.0	10.98	2.13	3.41	2.60	2.92	250	16.7	35.7
Minimum	9.8	8.8	0	9.0	10.1	4.10	1.09	1.99	2.12	2.43	130	4.2	15.4
REPRESENTATIVE FLOURS													
Thatcher Minot, 1944	12.9	13.1	8.0	64.0	13.4	6.40	1.87	2.85	2.28	2.57	220	14.6	32.4
Durum, 1943	14.9	26.1	7.0	14.0	18.0	10.98	1.49	3.41	2.12	2.52	190	5.1	15.4
Tenmarq, 1944	9.9	25.5	6.0	31.0	12.1	6.22	1.39	2.16	2.33	2.59	130	12.0	31.4
Chiefkan, 1943	12.8	14.3	6.0	38.0	13.4	7.02	1.62	2.50	2.26	2.54	170	9.4	26.9
Baart, 1943	9.8	33.7	8.0	13.0	12.5	6.30	1.39	2.46	2.35	2.61	210	4.8	17.2

tention of the authors to indicate that these supercentrifugates are necessarily typical for the varieties used.

The results found in these 25 patent flours of 9.8 to 14.9% protein content, milled experimentally or commercially, show very wide variations in the amounts of supercentrifugate obtained, the gelling property of this liquor, and its viscosity. In some cases, the total constituents of the liquor from different samples varied approximately twofold in amount. Soluble protein values varied somewhat less and carbohydrates somewhat more than the other constituents. Sodium chloride, which was an added ingredient in the dough, showed a variation which was greater than could be accounted for by the effect of the amounts of the other constituents. When the relationship of salt to water was calculated, a variation of 2.43 to 2.92% was found. If all the water in a dough were utilized in dissolving salt, the concentration would be 2.35%. If only the water added in making a dough from these flours of 12% moisture were utilized in dissolving salt, the concentration would be 2.67%. Hence, it would appear that in some of the doughs all of the added water plus a portion of the flour moisture is used in dissolving salt, whereas in other doughs less water than was added is utilized as a solvent. These variations can also be explained as the result of differences in amount of bound salt. The reasons for these wide differences in salt concentration are under further investigation.

The force required to puncture the gluten in the Baker apparatus is reported in all of this work on both crude and purified glutens to help characterize the flours. A variation of 4 to 1 in resistance to

puncture was obtained on the crude hand-washed glutens. These, upon purification by two dispersions in the Waring Blendor, become at least twice as resistant with an approximate 2 to 1 variation.

Although the summary data would suggest there is a relationship among some of the properties and ingredients, such as viscosity and the yields of liquor and of pentosans, inspection of the individual results obtained, but not shown here, does not support this hypothesis. Inspection of the results from individual flours indicates a lack of correlation between any of the other values. The frequent absence of the gelling property previously reported on centrifugates from 2 to 1 batters by Baker, Parker, and Mize (1943) is not confirmed by this present work. Among the 25 flours inspected here, where the concentrated extracts separated from doughs were tested, only one yielded liquor that was not gelled at the end of one hour. This flour, though it gelled at once when tested, liquefied during the hour. These results indicate that an undiluted concentration of the pentosans is required to determine gelling tendencies and that this property is present in all refined flours.

Results from Dough Operations

The method of separating the liquid phase from dough by supercentrifuging afforded an opportunity of studying the effect of the operations of breadmaking upon its constituents and properties.

For this purpose, from each of the same 25 flours, four different doughs were prepared with 73% absorption and 2% salt, as listed in the left-hand double column of Table II. The first dough, labeled

TABLE II

PROPERTIES OF SUPERCENTRIFUGATES FROM 25 FLOURS OF 1943 AND 1944

Dough operation		Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	-SH as G-SH	Gluten puncture	
How mixed	When centrifuged											Crude	Pure
		g		sec	%	%	%	%	%	%	ppm	g	g
Minimum 1½ min.	At once	23.8	6.5	30.8	13.1	6.61	1.55	2.61	2.33	2.62	180	10.2	28.2
More than minimum	At once	22.4	6.3	32.5	13.4	6.87	1.59	2.62	2.32	2.62	177	10.0	27.5
More than minimum	After 3 hr.	28.1	6.0	29.0	15.0	8.45	1.68	2.58	2.29	2.63	179	10.2	26.4
More than minimum with yeast	After 3 hr.	30.7	5.7	30.0	10.5	4.15	1.65	2.34	2.36	2.61	167	12.9	28.8

"minimum mix—at once," was mixed 1½ minutes, this being the minimum time required to wet completely the entire mass. This dough was then supercentrifuged at once. The second dough, labeled

"more than minimum—at once," was prepared by mixing as long as required for the optimum development of a dough from the flour at normal absorption. This procedure was used because of the extraordinary long mixing required at the 73% absorption by a few of the doughs which developed heat and also permitted considerable enzymatic action. The resulting doughs were thus all undermixed to varying degrees. The third yeastless dough was exactly like the second, with the same amount of mixing, but was allowed to stand 3 hours before centrifuging. The fourth dough was similar to the third except that it contained 2.5% yeast.

In comparing these results, the difference between the first and second doughs shows the effects of mixing; between the second and third doughs is shown the effects of autolysis in a yeastless dough upon standing for 3 hours. The fourth dough shows the effects of the yeast as distinguished from the autolysis in the third dough. A fifth comparison, showing the difference between the mixed dough centrifuged at once and the fermented 3-hour dough, gives the over-all inclusive effect during fermentation for 3 hours.

The dough operations do not alter appreciably the physical properties of the separated supercentrifugate except the quantity obtained. A large increase in the amount of the separated liquid phase of dough was found where yeastless doughs had stood 3 hours before centrifuging. A further increase was obtained where these doughs had been fermented. Tests, not reported here, show that most of the increase in liquor volume occurs in the first hour and is not altered by ordinary dough treatments. The action is somewhat similar to that observed in synaeresis and indicates by the unchanged composition that the dough materials pack more closely in the centrifuge after they have had a period of rest. The gel value and the viscosity are but little changed.

The changes in soluble solids are largely due to changes in carbohydrates shown in the succeeding column (Table II). The small increase in sugar occurring during mixing is doubtless due to the rapid attack of the diastatic enzymes upon broken starch granules. There was a further increase of 1.6% in the liquor during 3 hours. During fermentation a large portion of the total sugar in the liquor was consumed, averaging 4.3%.

Soluble pentosans show a tendency to increase in amount during mixing and standing, whereas yeast does not appreciably further affect them.

Soluble protein is substantially unaffected by all operations in breadmaking except fermentation, during which the yeast consumes some of the nitrogen materials in its metabolic processes. The

apparent decrease in 3 hours is due to the effect of carbohydrate increase on the percentage.

The amounts of salt given as percentage of the dough liquid are not clearly significant because the concentration is affected by the amounts of other materials present in the dough. Hence, calculation has been made as entered in the succeeding column, showing the percentage of salt in the water of the supercentrifugate. This relationship of the averages remains substantially constant throughout all dough operations and thus indicates that the changes in dough properties encountered in breadmaking are not due to changes in the amounts of free and bound water in the dough. This has been shown also by Kuhlmann and Golossowa (1936). The liquor from individual flours may show considerable effect from dough operations, but these variations are peculiar only to each flour and do not disprove the generalization from the averages that the handling of dough in breadmaking produces its effects on dough properties by other changes than can be found in the liquid phase.

The sulfhydryl compounds are not much affected by dough operations excepting by fermentation where a very definite decrease in their average concentration occurs. A few flours, however, show no change. Thus, the conclusions reported by Hullett and Stern (1941) were usually, but not always, confirmed.

Crude gluten is strengthened by fermentation, but other dough operations show no effect. However, the method of preparing crude gluten is uncertain, so that more conclusive indications possibly are to be found in the purified glutens. These results indicate that there is a small drop in the resistance of gluten to puncture both with mixing and time. This loss is more than recovered during fermentation.

Aging of Flour

The aging of flour is known to produce very definite changes in its baking properties. The method of separating the liquid phase in dough offers an opportunity to study the effect of age on the liquid phase. From 1943 wheat, 16 flours, either mill produced or experimentally milled, were available for the study of age. The flours were analyzed and then stored for a 6-month period at 1°C in closed metal containers. Probably, under this condition of storage, oxygen was partially replaced by carbon dioxide from respiration, as reported by Working (1936). The results are thus not entirely analogous to those obtained in ordinary commercial storage. In order to study the effect of age, supercentrifugates from the four dough operations were separated from these flours when fresh and after 6 months' storage. Table III gives these results. The effects of 6 months' storage can

TABLE III

PROPERTIES OF SUPERCENTRIFUGATES FROM 16 FLOURS OF 1943

Dough operation		Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	-SH as G-SH	Gluten puncture	
How mixed	When centrifuged											Crude	Pure
		g		sec	%	%	%	%	%	%	ppm	g	g
AS RECEIVED													
Minimum 1½ min.	At once	26.1	5.0	27.1	13.5	7.02	1.51	2.56	2.41	2.71	178	9.4	26.3
More than minimum	At once	25.8	4.9	25.6	13.8	7.27	1.54	2.59	2.40	2.71	178	9.4	27.1
More than minimum	After 3 hr.	31.4	4.1	27.4	15.7	9.09	1.67	2.56	2.38	2.71	190	9.4	26.6
More than minimum with yeast	After 3 hr.	33.6	3.8	24.6	11.3	4.96	1.62	2.32	2.40	2.70	168	13.5	27.3
AFTER 6 MONTHS' STORAGE													
Minimum 1½ min.	At once	27.1	7.0	24.0	13.5	7.14	1.48	2.54	2.34	2.63	173	10.0	25.9
More than minimum	At once	25.3	7.2	27.0	13.7	7.38	1.48	2.50	2.34	2.64	179	9.2	25.4
More than minimum	After 3 hr.	32.3	5.0	24.0	15.3	8.79	1.62	2.53	2.36	2.64	192	9.3	25.3
More than minimum with yeast	After 3 hr.	34.0	5.6	22.0	11.0	4.93	1.50	2.23	2.34	2.64	172	13.4	26.5

best be observed by comparing the values for each property or ingredient from the respective operations. The results indicate that the physical properties of the dough supercentrifugate were not greatly changed by flour storage with the exception of gel value, which became definitely stronger. The amount of carbohydrates found in the liquor from the aged flour was slightly less where the dough was permitted to stand 3 hours before separation, thus indicating that some diastase had disappeared from the flour during storage. There were also small decreases in the amounts of soluble pentosan and soluble protein. These decreases are particularly noticeable in the fermented samples. Possibly the alcohol from fermentation has a greater effect upon the solubility of pentosans of flours stored 6 months. Soluble protein is slightly more available for yeast metabolism in the aged sample. The most striking change in these doughs is found in the relation of salt to water; the aged samples furnish a more dilute salt solution, which indicates that there is either less bound water in these doughs or more bound salt. That the latter is more likely true is indicated by comparing the analyses of the fresh and aged samples mixed 1½ minutes and centrifuged at once. The constituents in these two sets are substantially identical in amounts, thus suggesting that no change in the water binding capacity has occurred over the 6-month storage

period. Thus, the lowered salt concentration would appear to be due to a decrease in the amount of salt in solution, indicating that salt has been taken into or combined with some of the other dough ingredients.

The sulfhydryl determinations show no change over the 6-month period. This surprising result is probably due to the samples being stored at 1°C in closed metal containers.

The resistance to puncture of the purified glutens in all operations shows a loss of about 1 g, indicating small decreases in gluten strength over the 6-month period.

Effects of Nitrogen Trichloride and Potassium Bromate

The action of nitrogen trichloride and potassium bromate was studied on a series of 15 flours produced from 1943 and 1944 wheats. The amounts of nitrogen trichloride used on these samples varied from 0.5 to 1.5 g per cwt, depending upon their effects in the baking tests. The amounts of bromate used varied from 10 to 30 ppm for the same reason. The analysis of liquor separated from fermented doughs only is reported. Tests were made on doughs from untreated flour, from flours treated with nitrogen trichloride, and also on similar sets of doughs to which bromate was added. These results are given in Table IV. The only appreciable change in the physical properties

TABLE IV

COMPOSITION OF SUPERCENTRIFUGATE FROM 3-HOUR FERMENTED DOUGHS TREATED WITH POTASSIUM BROMATE OR MADE FROM NITROGEN TRICHLORIDE TREATED FLOURS

(Average of 15 flours of 1943 and 1944)

Treatment	Liquor yield	Gel value	Viscosity	Soluble solids	Sugars by diff.	Soluble pentosan	Soluble protein	NaCl	NaCl in H_2O	–SH as G–SH	Gluten puncture	
											Crude	Pure
	g		*sec*	%	%	%	%	%	%	*ppm*	*g*	*g*
AVERAGE OF 15 FLOURS OF 1944												
Untreated	30.7	5.9	31.6	10.1	3.85	1.66	2.30	2.29	2.54	166	13.6	30.3
+Nitrogen trichloride	31.9	5.9	32.7	10.1	3.91	1.62	2.29	2.28	2.54	154	13.7	30.5
+Potassium bromate	31.2	6.1	30.6	10.1	3.88	1.64	2.29	2.29	2.55	159	12.6	27.7
+Nitrogen trichloride and potassium bromate	30.4	6.2	32.4	10.1	3.99	1.60	2.24	2.27	2.53	151	13.3	27.4

of the various supercentrifugates is a slight increase in viscosity in the nitrogen trichloride treated samples. This is hardly large enough to be significant. The amount of carbohydrates in solution clearly indicates that neither reagent has affected diastatic action in these doughs. This result is somewhat surprising and gives no explanation

for the changes in crust color sometimes observed when baking nitrogen trichloride treated flours. No appreciable action was found on soluble pentosans or proteins or the distribution of water and salt in the dough system. The sulfhydryl compounds were definitely lowered by the oxidizing agents, the nitrogen trichloride treatments giving the greater effect on these fermented doughs.

A more complete study of the action of nitrogen trichloride on sulfhydryl compounds in flour is shown in Table V. Here, the amounts

TABLE V

SULFHYDRYL COMPOUNDS IN SUPERCENTRIFUGATES FROM DOUGHS MADE FROM NITROGEN TRICHLORIDE TREATED FLOURS SEPARATED AT DIFFERENT STAGES OF DOUGH HANDLING

(Average of 15 flours of 1943 and 1944)

Dough operation		Untreated	NCl_3 treated
How mixed	When centrifuged	–SH as G–SH	–SH as G–SH
		ppm	*ppm*
Minimum	At once	177	179
More than minimum	At once	178	175
More than minimum	After 3 hr.	180	187
More than minimum with yeast	After 3 hr.	166	154

of sulfhydryl, reported as ppm of glutathione in the liquid phase, are shown for the dough operations used in Tables II and III. It is to be noted that the moderate treatments with nitrogen trichloride have produced no change in the sulfhydryl content of these 15 flours until they were fermented. The action of the yeast upon the untreated flours produced a decrease of approximately 14 ppm, whereas on the treated flours a decrease of 33 ppm was obtained. These results indicate that the action of nitrogen trichloride upon sulfhydryl compounds is to alter them in some manner so that yeast has a greater action upon them during fermentation. Baker, Parker, and Mize (1944) have previously shown that very heavy treatment with nitrogen trichloride lowers the soluble sulfhydryl content of unfermented dough.

The action of bromate upon the strength of gluten during fermentation is shown in Table IV. A loss occurred in resistance to puncture of about 3 g, amounting to 10%. This decrease confirms results previously published by Baker, Mize, and Parker (1943), which showed that bromate lowered the resistance of gluten to puncture.

Effect of Different Crop Years

The data presented show samples separately analyzed from the 1943 and 1944 crops. The top row of figures in Table IV labeled "untreated," and the fourth row in Table III labeled "more than minimum with yeast after 3 hr.," both on new flours, are directly

comparable. The liquor of the 1943 crop, as compared to the 1944, had more diastase, formed a stronger gel, had a lower viscosity, a higher concentration of salt in the water of the liquor, and yielded a weaker gluten on purification. These results do not necessarily mean that there was this difference generally in the two crop years, because there are not enough samples selected to establish this as a fact. They are compared here, however, as being suggestive of differences which may be encountered.

Baking Results with Supercentrifugate

Some evidence as to the action of flour solubles on dough properties can be obtained by the use of dough liquor as such to furnish the required water in breadmaking. The concentration of flour solubles in the dough is thus approximately doubled. These experiments were made with a northwestern spring wheat patent flour of 11.9% protein and 0.39% ash, using the straight dough process. It so happens that enough salt and sugar are present in the solution from this flour to supply the amounts needed for breadmaking. Hence, corresponding amounts of salt and sugar were added only to the doughs made with water. The excess materials present in the doughs made with supercentrifugate are soluble pentosans, proteins, sulfhydryl compounds, and enzymes. The total water added to the doughs was held constant regardless of whether water or supercentrifugate was used. Equal weights of dough were baked. The effects upon the properties of dough and bread are marked and readily observed and are summarized from tests on many other flours. Doughs become softer and more yielding though not overmixed in character. "Buckiness" disappears and in some cases the dough may even possess the character of excessive flow. Immediately after such doughs are mixed, excessive stickiness is observed, but this disappears during fermentation. They mold and shape more easily. During proof the doughs rise more rapidly but with less boldness. The excessive flow and surface stickiness produced by the supercentrifugate in the freshly mixed dough suggested that the absorption should have been decreased. The dryness of the dough when molded and the character of the finished bread indicated that even more water could have been used without damaging the bread had it been possible to handle the doughs at time of mixing.

When baked, the bread made with supercentrifugate has larger volume with greater oven spring and a richer colored crust, as shown in Figure 1.

The two loaves on the left were made with distilled water without and with bromate. Those on the right are corresponding loaves,

in which supercentrifugate supplied the same amount of water. It is to be noted that the latter loaves are larger with finer grain in both instances and gave a positive response to 20 ppm bromate. The control loaves, on the contrary, were distinctly damaged by this comparatively large bromate treatment. The liquor ingredients have modified or protected the dough in such a manner that bromate has produced an improvement without injurious effect. This protection is similar to the action of milk, as reported by Larmour, Working,

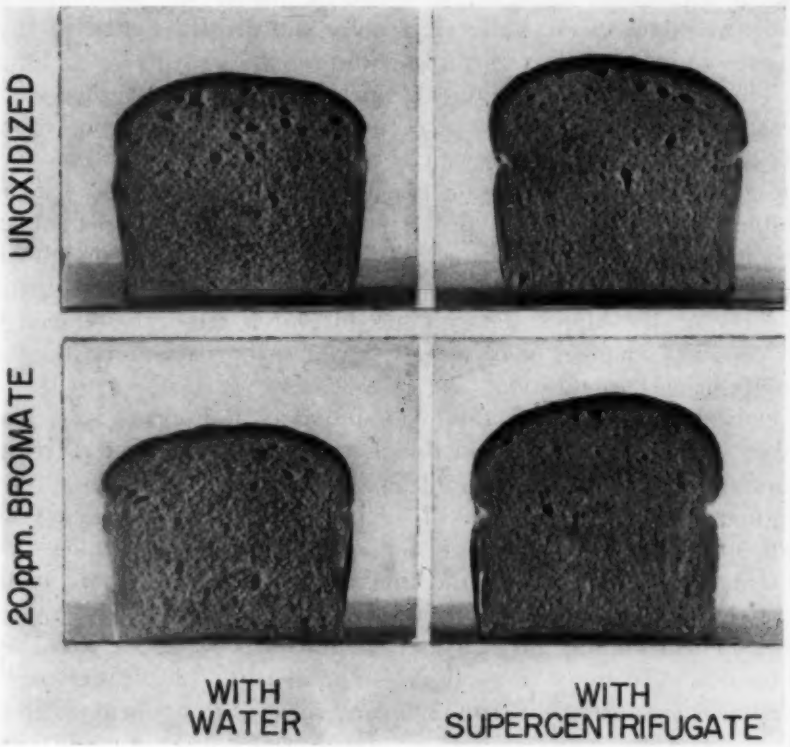

Fig. 1. Effect of supercentrifugate in breadmaking.

and Ofelt (1939) and later shown by Baker, Mize, and Parker (1943) specifically to protect gluten from damage. The improving effects obtained from supercentrifugate in these experiments are due to other ingredients than enzymes, for even better results have been obtained where the liquor has been heated to 80°C before use.

Discussion

In dough the liquor phase must be the medium through which all reactions occur during manipulation and fermentation. Gluten and

starch are permeated by and suspended in the liquid medium. The liquid phase becomes the medium through which changes occur, and dough properties are altered because only through it can additions or withdrawals of ingredients from gluten or starch take place. Thus, the analysis and examination of dough liquor should disclose or indicate the reactions that are responsible for dough changes.

Doubtless there remain other factors in dough liquor than those determined, such as enzymes, metabolic products of yeast, and protein and starch degradation products, which play an important role. The data given, however, largely indicate the extent of these changes and their influence can be interpreted.

The changes in dough liquor properties, which the average values presented here indicate, do not occur in all cases so that one cannot necessarily infer the changes which are going to occur in a given dough. The changes from fermentation are such as one would expect. Sugars, soluble protein, and sulfhydryl compounds disappear, alcohol and carbon dioxide are produced. A physical change occurs in dough with time that permits more liquid to separate. This plus the gelling property of the liquid and changes in gluten strength induced by the chemical changes may largely account for variations found in dough during fermentation.

One observation from this work indicates that proteolysis plays a very small part, if any, in dough reactions where sound, refined flours are used. No significant changes are found in soluble protein or gluten strength of doughs which have stood 3 hours without yeast. Fermentation, however, shows a relatively large effect upon these protein components, thus indicating that the changes found in fermented doughs are due to causes other than proteolytic enzymes from flour. The softening of yeastless doughs, which has so often been taken as a measure of proteolysis, is apparently a physical change in dough properties resulting from rearrangement of components. In the softened doughs the solids pack tighter in the centrifuge. All that is required to explain the softening change is the relaxation of the gluten micelle from the strained condition produced by mixing.

All sulfhydryl compounds exist naturally in an unreactive form in native protein and are measurable only after complete denaturation with guanidine hydrochloride (Neurath, Greenstein, Putnam, and Erickson, 1944). Unfortunately, the sensitivity of the method is too low to yield accurate results with such very low concentrations. Undoubtedly changes in sulfur have occurred which it was impossible to detect. Some flours showed no detectable change in any dough operation. This surprising result may, in part, be due to the weakness of the method but still shows clearly that some flours are very

resistant to changes in their sulfhydryls. In six fermenting doughs no loss of sulfhydryl was detected. This result indicates that the conclusions of Hullett and Stern (1941) may not be universally true.

The method of separating a liquor from dough in the supercentrifuge offers an opportunity of studying many other variables in flours and doughs not reported here. The effects of temperature changes and absorption or of other dough ingredients, such as sugar, milk, enzymes, etc., could well be studied by this method. Other treatments of flour and methods of handling dough might yield interesting results. The action of heat on dough could be studied up to the point of starch swelling.

Summary

A procedure has been described for the separation of a liquor from doughs of 73% absorption by means of the Sharples supercentrifuge operated at 40,000 rpm.

Supercentrifugates so obtained have been prepared from doughs made with 25 different flours subjected to four distinct dough operations. These liquors have been analyzed for soluble solids, pentosans, proteins, (–SH), and salt content, and have been tested for yield, viscosity, and gel strength. The analyses and tests show the comparative effects of mixing, 3-hour standing, and 3-hour fermentation upon all flours studied.

Little correlation was found between amounts of supercentrifugate ingredients themselves, between the amounts of liquor obtained and the composition of the liquor, or between properties of the dough liquor and concentration of its ingredients. The action of nitrogen trichloride and bromate has been shown to be largely due to either changes in physical properties of gluten or in soluble sulfhydryl compounds. Flour storage results in an increase in salt binding capacity. Dough operations before baking, as well as flour or dough oxidizing treatments, do not alter the water or salt binding capacity of flour substances.

Acknowledgment

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THE DISTRIBUTION OF WATER IN DOUGH

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Bread dough is produced by mixing together a solid and a liquid. When the two are combined to make dough the resulting mass has both solid and liquid properties. It is elastic, resists distortion, but will flow slowly with time. When enough water is added to a dough it becomes a fluid.

The theory usually accepted about the distribution of water in dough is that a large portion of it is free while the remaining portion combines with the flour constituents to form hydrates, or is bound by polar groups or otherwise reacted in such a manner that it is no longer available as a solvent. This theory of bound water has developed from the hypothesis advanced by Newton and Gortner (1922) and is in accord with Gortner's (1938) later views in respect to the state of water in biological fluids and in solutions of hydrophilic colloids. Alsberg (1927) quoted the earlier work of Rodewald (1896) and Boutroux (1897) who calculated that 82% of the water in dough is bound. Swanson (1943) estimated by a method of calculation similar to that of Boutroux that two-thirds of the water is bound. Kuhlmann and Golossowa (1936) found that the solids in flour bind 44.4% of their weight of water, as estimated by a refractometric method based

on that of Dumanski (1933). The freezing point method of Newton and Gortner (1922) for the determination of bound water was adopted by Skovholt and Bailey (1935) and Vail and Bailey (1940) for dough measurements. These workers found that flour substance bound respectively 45.4% to 42.1% and 28.6%. Swanson (1943a) states that since there is no sharp line of demarcation between free and bound water, variations in the method of measurement may be a considerable factor in the divergent values reported.

Baker, Parker, and Mize (1945) have described a liquor separated from doughs by means of the Sharples supercentrifuge. The concentration of salt in this dough liquor should be a measure of the amount of water available in the dough for dissolving purposes and hence of the bound and free water content of the dough under actual dough conditions. These authors found the concentration of salt in supercentrifugates when calculated in relation to the water present varied from 2.43% to 2.92% in different doughs, thus indicating a possible wide variation in the distribution of water in doughs.

Experimental

In order to study the distribution of water in a dough, a commercial flour was selected which gave dough liquor of approximately average salt to water ratio. This flour was a hard winter patent containing 12% moisture, 11.8% protein, and 0.40% ash, as found. It was made into dough by adding 73% of its weight in water and 2% of salt. The composition of the dough was as follows:

48.6	g of water
37.5	g of starch
6.1	g of gluten
6.66	g of flour solubles
1.14	g of sodium chloride
100.00	g of dough

This dough was supercentrifuged in the manner described by Baker, Parker, and Mize (1945) for varying periods of time to determine whether the liquor forced from the dough was of uniform composition.

In Table I are listed the amounts and analyses of the supercentrifugate obtained from five separate centrifugings of the dough at 10, 20, 40, and 60 minutes and also that amount obtained in 60 minutes plus all the liquid that could be removed from the surface of the dough by a rubber policeman after drainage had been completed. Supercentrifuging for more than an hour produced no further appreciable separation of liquid. It is to be noted that the separation of liquor from the dough is substantially completed in 40 minutes and that the composition of the free flowing liquor as regards the amount

TABLE I

CHARACTER OF SUPERCENTRIFUGATE SEPARATED AFTER VARIED LENGTHS OF CENTRIFUGING

Supercentrifugate period	Liquor yield	Water in liquor [1]	Concentration of NaCl in liquor	Concentration of NaCl in water in liquor
	g	%	%	%
10 minutes	24.6	87.6	2.34	2.60
20 minutes	30.8	87.6	2.35	2.62
40 minutes	35.9	87.7	2.38	2.64
60 minutes	36.7	87.4	2.34	2.61
60 minutes plus very viscous liquid	39.9	86.7	2.34	2.63

[1] The water in the liquor was determined by evaporation on a steam bath to constant weight.

of water was constant at all periods of centrifuging. When the viscous material was scraped from the surface of the dough an increase in solubles was obtained, which was largely pentosans, the amounts of which are not reported here. The total amount of liquid removed in the experiment represented approximately one-half the water added in making the dough.

It is to be noted that irrespective of the time of centrifuging or the scraping, the relationship of salt to water was substantially constant in the successive portions of liquor, thus suggesting that the liquid remaining in the dough was of the same composition.

The amount of water used in making this dough, i.e., 73 g plus the 2 g of salt added, would make a solution containing 2.67% of salt. Liquor separated from the dough contained only an average of 2.62% of salt, thus suggesting that there is more free water in dough than was added in making the dough and indicating that either some of the flour moisture is present as free water or some of the salt is combined with dough ingredients and removed from the liquid system.

Action of Starch and Gluten. In order to find how much water the insoluble ingredients of flour may bind, experiments were conducted on starch and gluten under conditions simulating those in dough. Starch was washed, purified with distilled water, vacuum dried, and then studied for its water-binding characteristics by exposing to the vapor of a 2.62% sodium chloride solution and then determining the total amount of moisture in the starch after equilibrium was reached. Under these conditions, 100 g of starch contained 32.6 g of water at 85°F (29.4°C). In a similar experiment in which the starch was exposed over the supercentrifugate from the dough, it contained 30.1 g of water.

Similar experiments were conducted with gluten purified by dispersing it four times in 0.15% sodium chloride solution, as described

by Baker, Mize, and Parker (1943). This purified gluten was dried in vacuum at 45°C and ground to 40 mesh. When suspended in the vapor of 2.62% salt solution, 100 g of gluten took up 40.9 g of water and when suspended over supercentrifugate from the above-described dough, the gluten absorbed 39.3 g of water. These results are summarized in Table II.

TABLE II

WATER BINDING ACTION OF STARCH AND GLUTEN

	Water
WATER BOUND BY 100 G OF DRY STARCH AT 85°F (29.4°C)	
From Vapor	
Over 2.62% sodium chloride solution	32.6 g
Over dough liquor	30.1 g
WATER BOUND BY 100 G OF DRY GLUTEN AT 85°F (29.4°C)	
From Vapor	
Gluten over 2.62% sodium chloride solution	40.9 g
Gluten over dough liquor	39.3 g
WATER BOUND IN 100 G OF DOUGH AT 85°F (29.4°C) (CALCULATED)	
37.5 g of starch binds	11.3 g
6.1 g of gluten binds	2.4 g
Total bound water in dough	13.7 g

The amount of water bound in 100 g of this dough at 85°F (29.4°C) by starch and gluten can be calculated from the results obtained by suspending these ingredients in the vapor of dough liquor. This indicates that starch binds 11.3 g of water and gluten binds 2.4 g, giving a total of 13.7 g of water bound in the insoluble ingredients of dough. It would appear reasonable that the amount of water which these ingredients absorb when exposed to the vapors of dough liquor is the same as they take up in the dough itself, where they are immersed in a similar liquid, because the vapor pressure conditions are identical whether in the vapor or in the liquid and the equilibrium should be the same in both cases. It is possible that the small amount of salt which it was necessary to leave in the gluten for its preparation may cause it to absorb a little more moisture than would be taken up under actual dough conditions where no such excess of salt would be present.

If the indicated 13.7 g of bound water be calculated to the basis used by Skovholt and Bailey (1935), 100 g of dry flour binds 27.3 g of water. Vail and Bailey (1940), when working with salt-free doughs at freezing temperatures, determined that 100 g of dry flour binds 28.6 g of water. These similar results suggest that bound water in dough is not greatly altered by salt and temperature.

Effect of Salt. The effect of salt on the amount and composition of the supercentrifugate obtainable from dough was tested by making doughs with their salt content varied from no salt up to 4% and supercentrifuging for 20 minutes. The amount of liquid, viscosity, total solids, and salt content was determined. The results are shown in Table III.

TABLE III

SUPERCENTRIFUGATE FROM 200 G OF DOUGH WITH SALT CONTENT VARIED, CENTRIFUGED 20 MINUTES [1]

NaCl used per 100 g flour	Yield	Viscosity	Soluble solids	Sodium chloride	Salt-free solubles [2]
	g	*sec*	*g/100 g*	*g/100 g*	*g/100 g*
None	17.7	43	10.4	0.09	10.3
0.125 g	23.4	31	10.3	0.17	10.1
0.25 g	24.6	31	10.8	0.35	10.4
0.50 g	28.5	25	10.7	0.64	10.2
1.00 g	31.2	23	11.4	1.30	10.2
2.00 g	30.7	25	12.5	2.61	10.2
4.00 g	27.1	27	14.3	4.63	10.2

[1] Methods of analysis used are described by Baker, Parker, and Mize (1945).

[2] Calculated by formula $\frac{\text{solubles} - \text{salt}}{100 - \text{salt}}$ = salt-free solubles in 100 g of salt-free liquor.

The concentration of solubles in the liquor was calculated to its concentration in the water of the liquor, as shown in the last column. The uniform values obtained indicate that varying amounts of salt in dough have no effect upon the total amount of flour substances dissolved in the liquid phase. This result probably indicates why bound water determined in the presence of salt checks so closely with that of Vail and Bailey (1940), where no salt was used.

We have not been able to determine the effect of low temperature on the composition of supercentrifugate because of difficulty in holding lower temperatures in the Sharples machine. Neither was it possible to study absorptions at 60% as used by Vail and Bailey (1940), since little liquor separates from such dough. The similar results obtained by these different methods further suggest that the amount of water bound in dough is not greatly influenced by excess added water. The results of Baker, Parker, and Mize (1945) show that salt concentrations in the water of dough supercentrifugates vary from 2.43% to 2.92%. Hence, the agreement between our results and those of Vail and Bailey may be accidental and no definite conclusions can be drawn from the similarity.

Volume Relationships of Dough Phases. The gravimetric data on the bound water of dough do not clearly show its relation to the properties of dough. The relative volumes of the various dough phases

should give a clearer picture of their relative action to each other in breadmaking. The volumes can be calculated from their amounts and densities. The same dough was used, the composition of which is given in the first part of this paper. The results of these calculations are shown in Table IV.

TABLE IV

CALCULATION OF PHASE VOLUMES IN 100 G DOUGH OF 73% ABSORPTION

Dough substances	Weight	Density	Calculated volume	Calculated phase volume	Calculated phase volume
	g		*ml*	*ml*	%
Dough 73% absorption	100.0	1.235	81.0	81.0	100.0
Starch dry	37.5	1.530	24.5		
Water bound in starch	11.3	1.000	11.3		
			35.8		
Hydrated starch				35.8	44.2
Gluten dry	6.1	1.300	4.7		
Water bound in gluten	2.4	1.000	2.4		
			7.1		
Hydrated gluten				7.1	8.8
Total hydrated insolubles	(35.8 + 7.1) =			42.9	53.0
Total liquid	(81.0 − 42.9) =			38.1	47.0
Liquid and gluten	(38.1 + 7.1) =			45.2	55.8

The last column gives the space occupied by the different phases in percent of total dough volume. Hydrated starch occupies 44.2%, hydrated gluten 8.8%, and the liquid fraction 47%. The combined volumes of the hydrated gluten and the liquid which together surround the starch amount to 55.8%.

Tightly packed wheat starch from this flour was determined to have a density of approximately 1.13, as packed. Since the density of starch is 1.53, the voids in this packed wheat starch are calculated to be 26% of its volume, or 11.5% of the dough volume. If the granules of starch were of one uniform size, the voids would have been 50% of its volume. The lower figure of 26% is obtained because starch contains a large proportion of very small granules which pack in between the larger granules and occupy the voids (Stamberg, 1939). Thus, there is required only about one-fifth of the gluten dough liquor mixture to fill this minimum of voids. Such an assembly would be substantially rigid because the starch granules would contact each other. The gluten and liquid mixture in dough, by its volume, holds the starch granules apart and the mixture becomes fluid. The starch granules are the solid bodies around which the gluten liquor mixture

must move in dough as it responds to applied stresses. However, many of the smaller starch granules are tightly bound to gluten and must move with it in dough, thus producing an additional increase in resistance to distortion by dough.

When less water is used in making a dough, the concentration of the soluble solids in the liquor becomes greater and its viscosity increases. The gluten particles are brought closer together and the starch granules come nearer to each other. A marked increase in dough resistance to flow results. This crowding together of the gluten forces it into more contacts and thus results in quicker gluten reaction during mixing of doughs of less absorption. This is shown in the quicker rise of a mixogram curve, where less water is used.

Dough Liquor and Gluten Mixture. Calculations of Table IV show that 55.8% of the volume of this 73% absorption dough consists of gluten and liquid. The properties of such a mixture depend largely upon the amount and character of its gluten. The dough liquor plays a role in modifying these properties. This can be observed when gluten is washed from a dough by using starch-free dough liquor as the washing fluid. A great deal of care is required under this condition to hold the gluten together. A large soft mass of gluten impregnated with dough liquor is thus obtained which may possibly have characteristics similar to the mixture as it exists in dough. It is elastic but flows with astonishing ease under strain. It cannot be held in one piece when worked and squeezed as ordinary gluten but must be handled very gently. When centrifuged, liquor is forced out and the mass becomes firmer. If transferred to water its character is quickly changed to that of ordinary gluten as the solubles are removed. This is an irreversible process since ordinary gluten does not become softer upon working in dough liquor.

The strength of gluten increases when centrifuged or washed. Both processes enable gluten contacts and bonds to be established more readily. The maximum strength of gluten should thus be obtained when no free liquid or dough solubles are present. This condition is produced by exposing dry purified gluten to water vapor as already described. Finely ground gluten when it has hydrated to a state of equilibrium becomes cohesive, soft, plastic, and very strong, but has no quality of wetness. Such gluten possesses maximum strength and resistance to flow. It has no capillaries to hold a liquid phase and no pentosans or other solubles to lubricate or interfere with its cohesions.

Discussion

In view of the large amount of liquid present, it may be informative to consider bread dough as an aqueous medium which has its properties modified by the presence of flour. Flour batters are liquids. The addition of more flour converts them to dough. The fluid property of the batter remains in the dough. About three-fourths of the total water may remain as a viscous, liquid solution of the solubles. Starch binds a portion of the water, occupies about 44% of the gas-free dough volume, and constitutes the solid portion of the dough. Hydrated gluten, though largely fluid in its action, also has elastic properties from its cohesions and holds the fluid mass together. Dough thus contains a highly viscous liquid rendered slightly elastic by the bonds between the gluten micelle dispersed in the liquid. These properties are modified by an approximately equal volume of suspended starch which adds puttylike properties to dough.

Summary

When supercentrifuged for increasing periods of time, bread dough containing salt, but no yeast, yielded increasing amounts of liquid containing identical percentages of salt. This offered an opportunity for determining the amounts of free and bound water in doughs by analyzing the separated liquid for its salt and water content. With some flours the result indicated less bound water present in dough than there was moisture in the flour. This appeared improbable and suggested the method did not yield the correct result.

Therefore, the water-binding capacities of starch and gluten were separately determined by exposing purified salt-free samples to the vapor from dough supercentrifugate. When equilibrium was reached, the amounts of water absorbed were calculated. This indicated that the dry flour in dough binds 27.3% of its weight of water. This result is nearly the same as that found by Vail and Bailey (1940), who worked with salt-free doughs at 0°C.

The effect of salt on the distribution of water in dough was tested by preparing supercentrifugates from doughs of varying salt content from 0% to 4%. The addition of salt did not alter the concentration of flour solubles in the water of the separated solution, thus showing that salt in these concentrations does not alter the amount of free water in dough and hence also has no effect on the amount of bound water. This indicates that both Vail and Bailey's freezing point experiments and those of the authors with salt-free materials suspended in vapor give approximately correct results for the percentage of

bound water in doughs containing salt. The volumes occupied in dough by the solution containing solubles and by the hydrated insolubles, gluten, and starch were calculated from their known amounts and densities. In this dough of 73% absorption, 47.0% of its volume was aqueous solution, 44.2% hydrated starch, and 8.8% hydrated gluten. That hydrated gluten and the aqueous solution are closely associated and move together in dough around the starch was shown by removing the starch from dough by a gluten washing procedure using starch-free supercentrifugate as the washing liquid. The final, tender separated gluten was impregnated with the dough liquid in amounts similar to those in dough. It would appear that such a mixture, which together comprises 55.8% of the dough volume, surrounds the starch and contributes fluid and elastic qualities to dough while the starch adds puttylike properties to the dough.

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GAS OCCLUSION DURING DOUGH MIXING

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When studying the mixing of doughs in vacuum and in the presence of various gases, Baker and Mize (1937) observed that gases are progressively occluded in doughs during mixing. They measured the amounts of gas dispersed in a dough by comparing its density to that of the same dough mixed in vacuum. Doughs mixed in vacuum contained no voids or occluded gas and had a density of 1.24, whereas those mixed in gas had densities as low as 1.02 when given excessive mixing. Thus the overmixed dough contained gas equal to approximately 20% of the volume of the dough substance. At that time it was assumed that the stickiness and softness that often developed during excessive mixing might be explained by large amounts of occluded [1] gas. Later these same authors (1941) studied the origin of gas cells in bread dough and concluded that all cells found in bread originate from the operations which apply work to the dough, such as mixing, punching, rounding, and molding. It was shown that the yeast organism does not originate gas cells and that cells which are originated by work applied to dough may not be retained during subsequent operations unless the dough is in proper condition to avoid their loss.

The present study was prompted by the development of a simplified method for determining density whereby the occlusion of gases in dough could be rapidly followed throughout the course of the dough mixing period. The method was used to study the relationship between dough mobility, gas occlusion, and baking quality.

Method of Determining Density

The density of the dough taken directly from the mixer was determined by dropping small pieces into calcium chloride solutions of known density at 85°F (29.4°C) and observing whether the sample sinks or floats. Dough density can be readily determined by having a series of such solutions covering the range of densities encountered in dough mixing. The amounts of anhydrous calcium chloride required to prepare the solutions can be found in many handbooks. Calcium chloride solutions with densities varying between 1.24 and 1.00 cover

[1] In earlier papers the words "emulsed," "emulsification," etc., were used to describe the incorporation of gas into dough by mixing. This has here been changed to "occlusion," etc., as suggested by the Editor.

the densities of doughs encountered in these studies, including those which were too badly overmixed for handling, as well as those which were mixed in vacuum.

Figure 1 gives the necessary information for reading either the percentage of gas by volume or the volume of gas in 100 g of dough, as determined by the method described above. If fats or high absorption are used in dough, the densities of gas-free dough are lowered; hence, Figure 1 will give slightly high indications of the amount of gas in such doughs. Low protein flours, on the other hand, give the opposite effect. Care is required in selecting the dough samples for this determination as large bubbles may accidentally be incorporated in the dough and give misleading figures. Also, doughs of low density,

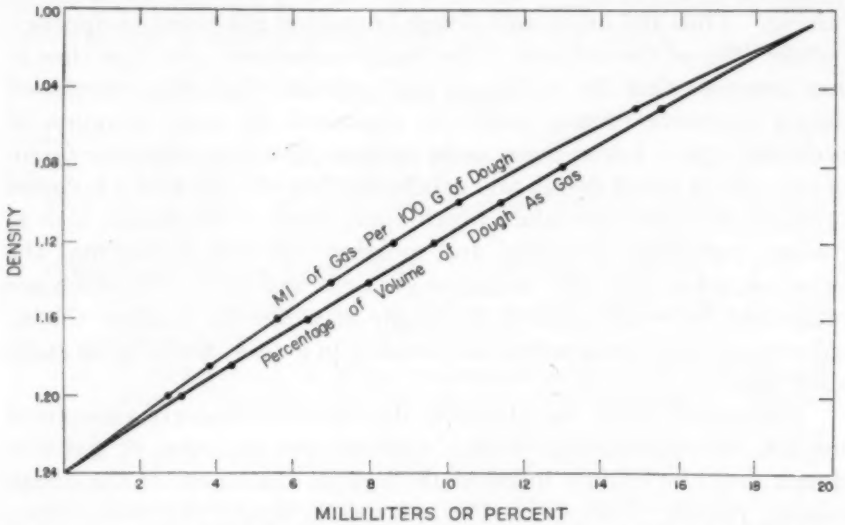

Fig. 1. The relation of dough density to the volume and percentage of occluded gas in average bread doughs without shortening.

such as produced by overmixing, must be very carefully handled or gas will escape. Apparently such overmixed doughs contain gas in fairly large bubbles which are very easily eliminated by manipulation. When handling doughs containing yeast, it is necessary to determine the density as soon as the mixing is stopped.

Materials

Unless otherwise stated, four flours, Hard Spring Patent, Turkey, Chiefkan, and Durum, were used for the experiments. Two are of good and two of inferior breadmaking quality.

Doughs containing 340 g of flour, 2% salt, and 4% sugar were mixed at optimum absorption in carbon dioxide in the McDuffy bowl

TABLE I

CHEMICAL AND BAKING CHARACTERISTICS OF FOUR TYPES OF FLOURS

Flour	Protein (12.0% moisture)	Ash (12.0% moisture)	Mixing time	Absorption	Vol. of 1 lb. loaf	Gluten puncture [1]
	%	%	*min*	%	*cc*	*g*
Hard spring patent	12.9	0.39	10	68	2830	29.4
Turkey	12.3	0.48	8	66	2715	25.4
Chiefkan (1941 crop)	12.7	0.40	3	67	2200	18.6
Durum	12.0	0.61	4	65	1800	16.0

[1] See Cereal Chem. 20: 506–516 (1943) for the method of determining gluten puncture.

at 65 rpm with the Hobart mixer. The optimum absorption of each flour was determined in a previous mix. To some doughs 40 ppm of sodium chlorite was added. Yeast was omitted in some cases to facilitate the density determinations. Its presence would not have materially altered the results unless testing was delayed. For experiments in the mixograph, doughs of the same relative composition formed from 35 g of flour were used. Carbon dioxide, which was released above the dough during mixing to eliminate the effects of oxygen, was retained by extending the mixograph bowl 1½″ upward with a thin sleeve.

Experimental

Dough Densities. Figure 2 gives the densities obtained by mixing the four flours (Table I) in the McDuffy bowl.

In doughs from the flours of good bread baking quality, gas dispersed very slowly both before and after the normal mixing requirement. The inferior flours occluded gas rapidly from the beginning of the mixing period. Much larger amounts of gas were occluded in the doughs made with these inferior flours, suggesting that excessive occlusion is not desirable and indicating that resistance to gas occlusion is one of the qualities of strong bread baking flours. This slower rate of occlusion of gas with the better bread flours could be interpreted to indicate that since such flours yield finer crumb which have more gas cells, the mixing causes an increase in the number of cells by gas subdivision rather than by occlusion. The resulting more numerous and smaller bubbles could be more readily surrounded by gluten and thus gastight bubbles be obtained, as suggested by Baker (1941).

Relation of Gas Occlusion to the Mixogram. Since gas occlusion is a foam-forming process, the changes encountered in dough mixing could be considered analogous to those in egg beating, with a similar stiffening and thinning taking place. This analogy suggested that the shape of

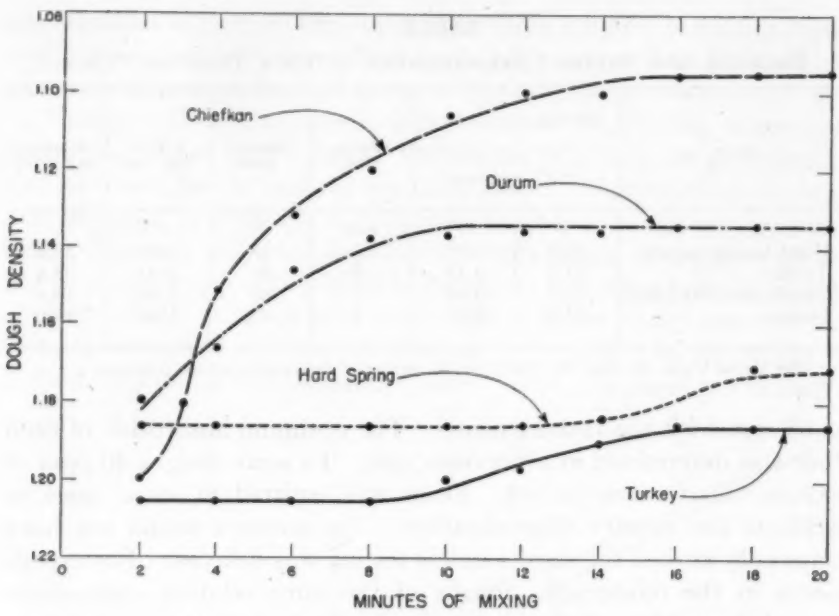

Fig. 2. Densities of unfermented, untreated doughs mixed in a McDuffy bowl in carbon dioxide.

a mixogram curve, due to changes in dough consistency, might result from gas occlusions. To test this hypothesis, densities were determined during the making of a mixogram. The relation between gas occlusion and dough consistency was thus observed.

The flours used in Figure 3 were the same as those listed in Table I. The densities of doughs were obtained during the course of the mixing operation of the mixograph by stopping the machine at a desired interval, removing a test sample, and determining the density. This procedure was repeated from the beginning on further samples of the same flour to determine other density values. Sufficient doughs were mixed to determine the densities throughout the entire mixogram. The densities of the doughs from each flour are plotted directly on the mixogram using the same time intervals on the abscissa but different units for density on the ordinate.

The results show that gas occlusion occurs more rapidly and somewhat more extensively in the mixograph than in the McDuffy bowl. The action in both mixers on different flours, however, is similar. The amount of gas occluded with time of mixing by these flours, with the exception of durum, is again in the reverse order of their bread baking quality, i.e., the more desirable bread baking flours show greater resistance to gas occlusion than do flours of poor baking quality.

The rate of gas occlusion increases slowly as the mobility curve first rises. When resistance to mixing reaches a maximum, the rate of occlusion greatly increases and gas is incorporated into the dough very rapidly. Thereafter gas occlusion again proceeds slowly. This continuing occlusion may be due, in part, to a rise in the temperature of the dough during mixing. The relative behavior of the mixogram and the gas occlusion curve suggests that the rise of the mixogram is due to gas occlusion. To further test this hypothesis, flours were mixed with 40 ppm sodium chlorite added to the liquor prior to combining with the flour, and the mixograms on the right side of

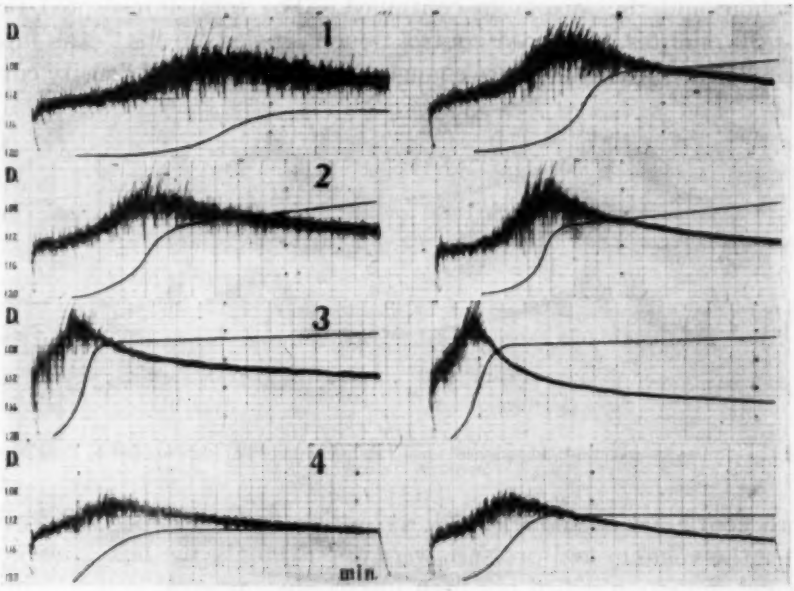

Fig. 3. Mixograms and densities of doughs made without (left side) and with sodium chlorite (right side).

Figure 3 were thus obtained. The oxidation caused a more rapid rise of the mixograms and a more rapid occlusion rate. The maximum rate of occlusion again coincides with the peak of the mixogram, suggesting, as before, that the rise in the pattern may be due to gas being incorporated in the dough.

If the increase in dough resistance as measured by the mixograph is due to occluded gas, then the resistance to mixing should decrease upon removing the occluded gas from the dough and increase again when the mixing is renewed. Most of the occluded carbon dioxide bubbles in dough can be collapsed and thus destroyed by subjecting the dough to pressure, thereby driving the gas into solution. If too

much gas is present for accomplishing the desired collapse of the bubbles by pressure, it can be more completely eliminated by first placing the dough in a vacuum, then under pressure. The elimination of occluded gases is shown by a return of dough density to its initial value.

Figure 4 gives the results of these experiments on chlorite-treated doughs. The mixogram labeled "Normal Mix" and "75 pounds pressure" was interrupted at the point where optimum "no-dough-time" baking results were obtained (Baker and Mize, 1941). The bowl containing the dough was removed from the mixograph, subjected to vacuum and pressure as above described, then returned to the machine and the mixogram continued. The total interrupted time was 30 minutes. When a control dough labeled "0 lbs." was interrupted, but without pressure changes, the mixogram and density curve

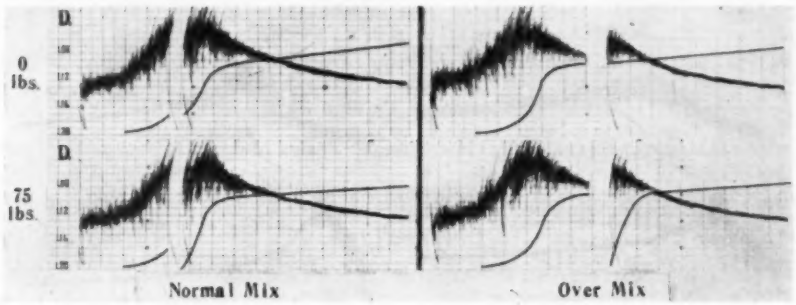

Fig. 4. Mixograms and densities obtained when vacuum and pressure were applied to doughs from interrupted mixograms.

both continued as though no interruption had occurred. However, after the vacuum and pressure were applied, only the mixogram continued as though it had not been interrupted, whereas dough density was returned to its original value of 1.20. Upon the resumption of mixing the density dropped rapidly as gas occlusions were quickly renewed. Thereafter density changes were substantially the same as those of the uncompressed dough. These two experiments indicate that the rise in the mixogram is not due to occlusion of gas. To further test this point, the experiment was repeated on a dough interrupted after the peak of the mixogram, and after the rate of occlusion had passed its maximum. These results are also shown in Figure 4. Here again, the pressure affected the density only. It is now clear that occlusion is only an accompanying phenomenon of mixing. The thickening or thinning of the dough during mixing is not due to occluded gas. The occlusion of gas always increases with the rise in the mixogram, and is fastest at its peak, after which further occlusion

continues slowly. Thus, apparently, the phenomenon causing the mixogram to rise to a peak also causes the dough to occlude gas. This coincidence of gas being trapped in dough at the time of maximum resistance to mixing suggests that in the formation of gluten during mixing, bubbles are caught in the connected gluten micelles as they cohere.

Relation of Gas Occlusion to Baking. Baking experiments using the "no-dough-time" technique have indicated that the best bread is obtained just before the rapid rate of gas occlusion occurs. To test this relationship micro-loaves were mixed in the mixograph and baked by a technique similar to that described by Van Scoyk (1937, 1939), but modified to the "no-dough-time" method using 40 ppm sodium chlorite, described by Baker and Mize (1941). The operation of the mixograph was stopped, the dough removed and immediately panned, proofed, and baked. Five doughs were mixed to the following degrees, respectively: the minimum (1½ minute mix), the optimum baking value (4¼ minute mix), the peak of the mixogram (5 minute mix), beyond the peak (5½ minute mix), and badly overmixed (12 minute mix). The mixogram, the densities, and the loaves from these five doughs are given in Figure 5.

The minimum mixed loaf indicates a lack of sufficient occluded gas nuclei to yield a fine cellular structure; hence, the very coarse grain.

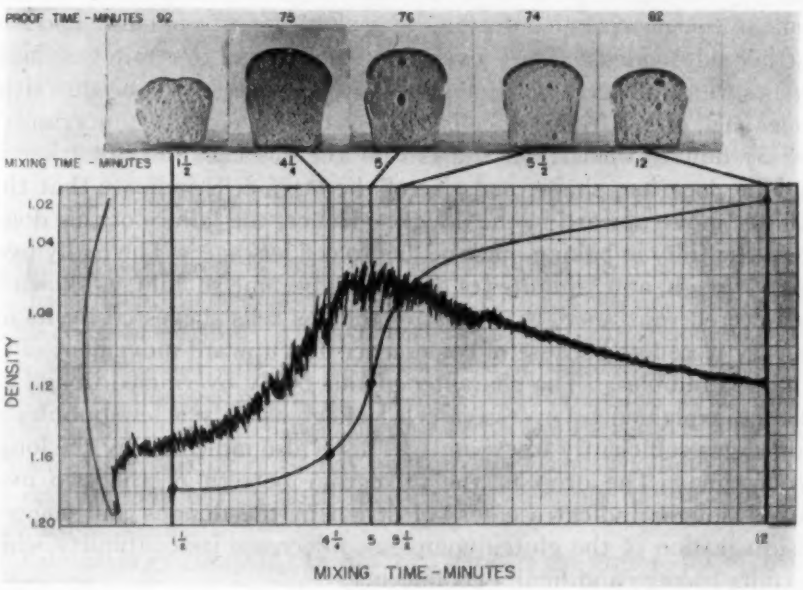

Fig. 5. Micro-loaves panned at once and baked from mixogram doughs at successive intervals of mixing with 40 ppm sodium chlorite.

Further, its long proof time and small volume indicate that the cells present failed to retain gases sufficiently to give normal proof or oven spring. The optimum loaf shows large volume with very fine uniform grain and absence of large bubbles. This loaf was taken just prior to the sharp decrease in density which occurs at the peak of the mixogram, and shows that at this point ample gas has been occluded for breadmaking and that it is divided into many gastight cells of small size. The loaf baked at the peak of the mixogram with 5 minutes of mixing shows the presence of large bubbles dispersed among the finely divided cells observed in the previous loaf. This cell development suggests that the gas rapidly occluded in the additional one-half minute to the peak of the mixogram was not all divided into small cells of uniform size. The larger cells expand during proofing but do not coalesce or rise through the dough because of the highly oxidized nonfluid character of these "no-dough-time" doughs, as reported by Baker, Parker, and Mize (1942). This loaf illustrates why the mixing period in making "no-dough-time" doughs is very critical. It has always been observed that the mixing must be stopped at a definite interval of time to obtain optimum results, and that further mixing results in irregular grain and cell size.

The remaining loaves show marked loss in volume from mixing beyond the mixogram peak. The one which was mixed 5½ minutes has very fine grain in addition to some large bubbles, indicating that some of the gas occluded during the peak of the mixing curve had been further subdivided. These overmixed doughs fail to retain gas during oven spring. The reason for this loss of volume must be due either to leakage of gas from the cells or to coalescence. The fine crumb of the 5½ minute mixed loaf shows that the gas cells did not coalesce, and the location, shape, and size of the large cells indicate that they did not move upward in the dough. Hence, the failure of this dough to spring fully in baking must be due to cell leakage. The badly overmixed dough, at 12 minutes of operation, became so fluid, as shown by its lowered resistance, that most of the gas cells suggested by its low density were lost because of coalescence and upward movement of the enlarged bubbles. The character of this badly overmixed dough can also be explained by excessive cell leakage as only a few bubbles retained gas sufficiently to expand. This is also indicated by the longer proof time. The drop in the mixogram pattern of the two overmixed doughs indicates a loss of strain in the doughs and suggests disintegration of the gluten complex to decrease its continuity which permits leakage and finally coalescence.

To show the effects of mixing without oxidation, the above experiments in baking bread at different stages of the mixing period were

repeated with doughs mixed in the McDuffy bowl. The undermixed, optimum-mixed, and overmixed doughs only were prepared because it is difficult when operating the McDuffy bowl to pick that point in the mixing which corresponds exactly with the peak of the mixogram.

Figure 6 gives two sets of three loaves each mixed in this manner. Both sets were made by the "no-dough-time" technique, one being oxidized with 40 ppm sodium chlorite, the other without oxidation. The breads made from oxidized doughs, when mixed in different amounts in the McDuffy bowl, show the same characteristics in grain and have similar dough density to the corresponding mixograph doughs, and thus suggest the number of cell nuclei observed is similar

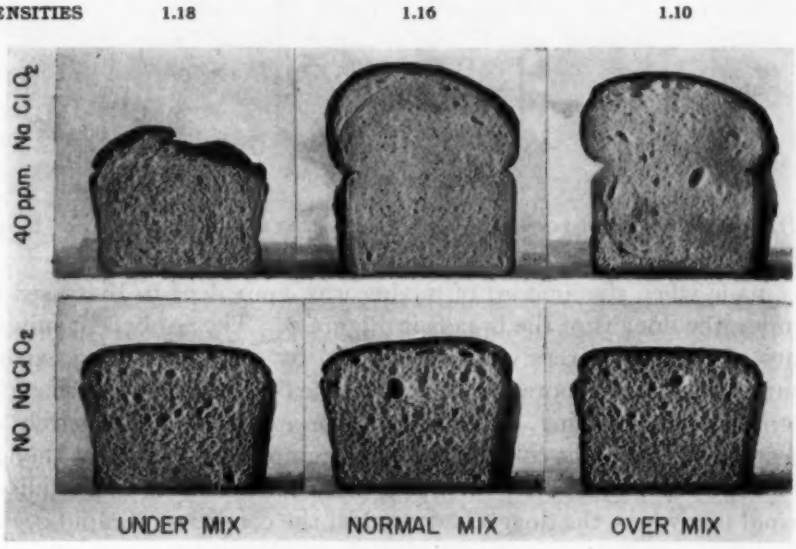

Fig. 6. "No-dough-time" doughs with and without sodium chlorite panned at once after different periods of mixing in a McDuffy bowl.

to the loaves mixed in the mixograph. When no sodium chlorite was used, identical loaves of very green character were obtained with all amounts of mixing. The densities of the unoxidized doughs, however, were substantially the same as those of the oxidized doughs at the different stages of mixing. The unoxidized bread fails to show any evidence of the gas occlusions indicated by the densities. The cells formed in the mixing must have disappeared from the dough during the proofing and baking of the bread by coalescence and upward and outward movement.

Relaxation of Doughs. In order to explain the apparent difference in the oxidized and unoxidized "no-dough-time" breads of Figure 6, similar doughs were formed in the mixograph. The operation of the

machine was interrupted at stated intervals for a period of one-half hour, after which the operation of the machine was resumed to continue the mixogram.

Densities of the doughs for all points determined were obtained by repeating the operation of the mixograph on separate doughs. These densities are plotted on the corresponding interrupted mixograms in Figure 7. The interruption of the mixograms in these two series

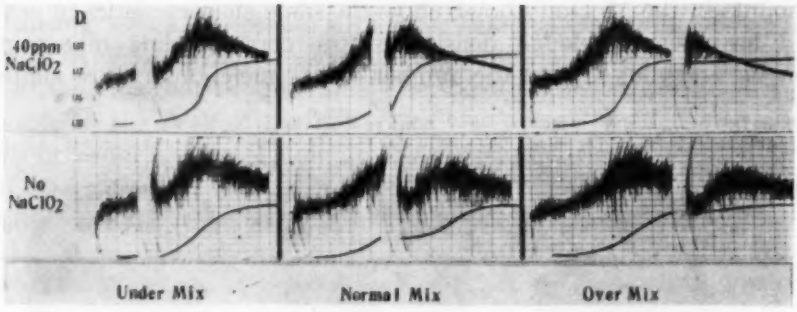

Fig. 7. Interrupted mixograms showing changes in dough mobility and density while resting. Doughs were similar to those used for Figure 6.

was made when the amount of mixing was equivalent to that used in forming the doughs of the breads in Figure 6. The equivalent mixing times of the two mixers were determined by separate baking experiments. It is to be noted that no occluded gas was lost during the interruption of mixing. Also, with one exception, the march of densities of the doughs continued upon resumption of mixing as though no interruption had occurred. The exception was in the unoxidized normal mix where the dough had reached the condition of rapid occlusion. This condition was lost during the rest period but was regained as the dough recovered its resistance to mixing. The mixograms of Figure 7 give an explanation for the marked difference between the "no-dough-time" breads of Figure 6 from doughs with and without sodium chlorite. In both series, the doughs resisted mixing and occluded gas in approximately the same characteristic manner, indicating organization of the gluten. Upon interruption, the doughs without chlorite were unable to retain the strain produced in the dough by the mixer. During the one-half hour standing these unoxidized doughs relaxed without losing occluded gas. Their resistance upon resumption of mixing was approximately the same as at the initial period of the mixing. The oxidized doughs, on the other hand, retained the mixing strain throughout the one-half hour standing and the mixogram resumed, in the case of normal or optimum mix, substantially as though the interruption had not taken place. Un-

doubtedly, the same property that causes the oxidized dough to retain mixing strain during standing also causes it to retain its occluded gas and cell structure during proofing and baking. Flow is substantially prevented; hence, coalescence or cell movement does not occur because the strain is not released. In the unoxidized dough the bubbles as they expand are unable to retain the strain, the dough structure relaxes, the cells coalesce and move through the dough; hence, the initial cell content created in the mixing is lost in the proof. This interpretation was also demonstrated in dough freezing experiments by Baker and Mize (1941). These experiments show that the effects of oxidation in breadmaking are obtained by reactions that retain the strains developed in dough while it is being worked.

Density of Sponge Doughs. The "no-dough-time" method of making bread used for all the work so far described herein is a useful tool for research. It differs from methods employed in practice in that all operations except mixing, proofing, and baking are eliminated. Further, it relies upon mixing to initiate all gas cells. It permits a minimum of enzymatic changes and fermentation products during the entire operation. The interpretations obtained from these "no-dough-time" breads cannot necessarily be applied to other methods of breadmaking. To study the mixing effects obtained with other methods of operation, the densities of commercial doughs were studied with the collaboration of four commercial bakeries. The results are listed in Table II.

TABLE II

DENSITIES OF COMMERCIAL SPONGE DOUGHS TAKEN AT THE END OF THE FINAL MIX [1]

Bakery	Dough density
A	1.130 (Average on 8 doughs) (With range 1.121 to 1.135)
B	1.130
C	1.115
D	1.120

[1] The authors wish to take this opportunity to express their thanks to Messrs. Gaston Dalby, J. H. Lanning, W. H. Cathcart, and R. T. Bohn for their collaboration in obtaining the densities on commercial doughs.

These values are considerably lower than any densities reported for optimum mixed "no-dough-time" doughs. To examine the reasons for this difference a comparative study was made of the dough density obtained in the McDuffy bowl with a 60/40 sponge dough and the density of an unfermented dough from the same flour.

The mixing of an unfermented dough with or without bromate in a McDuffy bowl gives the dough density results shown at the bottom of Figure 8. This density curve is similar to that obtained on this

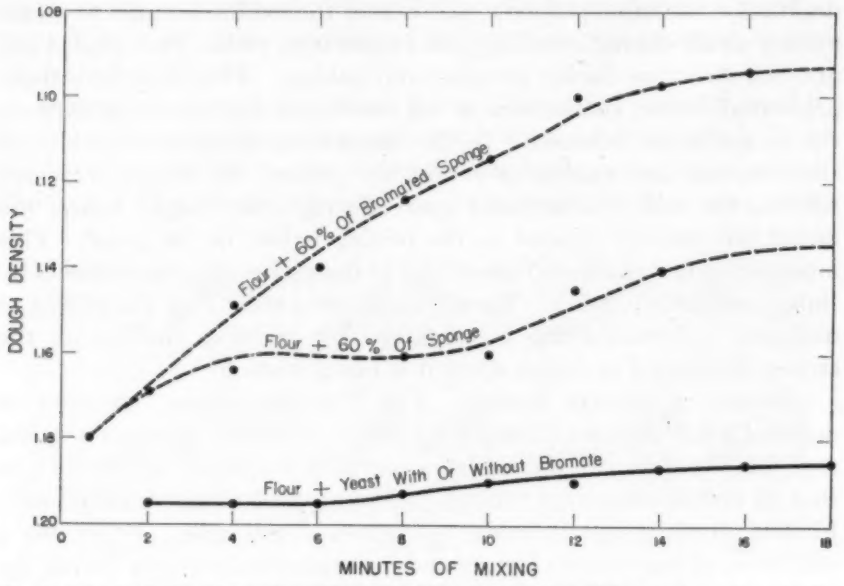

Fig. 8. Density during mixing of unoxidized and oxidized doughs formed from water, salt, and either flour and yeast, or flour and 60% sponge.

strong spring wheat patent flour shown as number 1 in Figure 2. It will be observed that bromate is without effect on dough density while assembling an unfermented dough. When undermixed doughs, as in a sponge, are permitted to ferment and then incorporated together with 40% new flour into a sponge dough, much greater occlusion of gas is obtained during mixing. Without bromate a density of 1.16 was obtained by mixing such dough to the optimum. When bromated sponge was mixed with the 40% new flour, a density of 1.125 was obtained by mixing to the optimum. This latter value is in the range of densities observed in commercial sponge doughs. These results indicate that fermentation markedly increases the rate of incorporation and amount of gas which dough occludes during mixing. Also, these results show that bromate increases the gas occlusion capacity of fermented doughs in a manner similar to the lesser action of sodium chlorite on unfermented doughs.

Summary

The amount of gas present in dough can be determined by comparing its density to that of a similar gas-free dough. Flours vary widely in the amounts of gas which they occlude during mixing. The slower mixing and better bread flours are more resistant. The rate at which gas is occluded during mixing varies widely during different stages of the mixing period, being slow at first and fastest at the time

the dough offers its greatest resistance to mixing. Dough densities plotted on a mixogram at successive time intervals suggest that the resistance to mixing is caused by the occluded gas. This was shown not to be true by removing occlusions of carbon dioxide gas by means of vacuum and pressure. With dough oxidized by means of sodium chlorite no change in the mixing resistance curve was produced by thus removing the occluded gas.

Undermixed doughs contain the smallest amount of occluded gas. Baking experiments with mixograph doughs show that in undermixed doughs the number of gas cells is small and they are leaky. This is shown by the high density, the coarse grain, small volume, and long proof time. The best bread is obtained near the peak of the mixogram just before the rapid rate of gas occlusion is reached. This fact, together with the fine grain of the bread, its large volume, and short proof time, indicates that the fine cell structure is obtained, not by gas occlusion, but by gas subdivision, and by organization of the dough and gluten, so the cells are gastight and retain their integrity throughout the breadmaking process. Further mixing rapidly incorporates gas to yield irregular-sized bubbles and nonuniform grain. Excessive overmixing results in loss of gluten continuity and coherence so that few cells are sufficiently gastight to expand and form the visible cell structure of bread.

The action of oxidation in improving doughs is not due to effects on gas occlusion but rather to preventing doughs from relaxing or flowing. Hence, oxidation of doughs prevents coalescence and bubble movement and results in retention of the occluded gas cells during their expansion in breadmaking.

Fermentation greatly increases the gas occlusion rate and capacity of doughs. Bromate further increases this effect. Commercial type sponge doughs mixed in a McDuffy bowl gave optimum doughs of the same density as a number of commercial doughs which were tested.

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EFFECT OF LEAF AND STEM RUST ON THE PHOSPHORUS METABOLISM OF HARD RED SPRING WHEAT [1]

A. HOFFER,[2] W. F. GEDDES,[3] and M. N. LEVINE [4]

(Received for publication April 18, 1945)

One of the objects of a cereal rust research program conducted at the University of Minnesota was to study the effect of leaf and stem rust upon the translocation of certain plant constituents into the developing kernel and on the chemical composition of the resulting grain. Geddes and Levine (1942) have reported the results of a study of the distribution of thiamine in Thatcher wheat during ripening in 1940. A similar investigation into the phosphorus content and its distribution in Thatcher and Marquis wheat, and the effect of rust thereon in 1941, is reported in this paper.

The literature on the phosphorus metabolism of wheat has been adequately reviewed by Miller (1939) in connection with his extensive studies on the chemical composition of the wheat plant at different stages of its development. Although several workers have studied the concentration of phosphorus, phosphorus content, and its distribution in the wheat plant at various stages of maturity, there is only limited information on the influence of rust infection on the phosphorus metabolism.

Most investigators have found that the actual amount of phosphorus present in the stems, leaves, and glumes decreases during the maturation of the plant; whereas the quantity of phosphorus present in the kernel increases. There is, however, lack of agreement on the trends in the total phosphorus content of the entire plant as it matures. Some investigators have found that the total amount of phosphorus in the plant reaches a maximum about the time of blossoming and thereafter decreases, presumably due to the return of phosphorus to the soil. Others report that some absorption of phosphorus occurs during its translocation into the grain, while still others have noted that the total phosphorus content of the plant remains relatively stationary from blossoming to maturity.

In his extensive studies in Kansas of the changes in dry matter

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[1] Joint contribution from the Division of Agricultural Biochemistry and the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. Paper No. 2254. Grateful acknowledgment is made for valuable assistance rendered by the personnel of the Work Projects Administration, Official Project No. 165-1-71-124, sponsored by the University of Minnesota during the year 1941.

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content and chemical composition of rust-free hard winter and soft winter wheat from the seedling stage to complete maturity, Miller (1939) found that phosphorus continued to be absorbed from the soil after heading and that the total amount of phosphorus in the plant did not generally reach a maximum until harvest. In general, the amount of phosphorus in the stems and leaves decreased, and that in the head increased, from the time of heading until full maturity.

Caldwell, Kraybill, Sullivan, and Compton (1934) studied the effect of leaf rust infection on the yield, physical characteristics, and chemical composition of winter wheat. Phosphorus determinations were made only on the grain itself at successive stages of development. They found that leaf rust produced no significant changes in the percentage of phosphorus present in either immature or mature grain. However, as severe rust infection reduced the head yield and acre yield, the total quantities of phosphorus per head were greater in the mature control plants.

Greaney, Woodward, and Whiteside (1941) studied the effect of stem rust on the productivity, chemical composition, and quality of mature Marquis, a hard red spring wheat variety. Like Caldwell *et al.* they found no significant difference in the percentage of phosphorus in the grain as a result of severe stem rust infection in 1934 and 1935. In 1937, however, the percentage of phosphorus in the grain appeared to increase with the increase in severity of stem rust infection. In their experiments, appreciable leaf rust infection occurred along with stem rust in the 1934 and 1935 series. The authors suggest that in the grain, "rust infection interferes considerably more with the deposition of organic than with inorganic compounds." In 1937, analyses were made of the mature straw in addition to the ripe grain. Severe rust infection resulted in a 50% increase in the percentage of phosphorus in the straw, in comparison with the relatively rust-free plants.

Materials and Methods

In the present investigation, Thatcher was used for the study of the effect of leaf rust, Marquis for that of stem rust. Both varieties were sown on May 5, plot treatments were started May 19, and continued through July 21. Inoculations with a uredial composite of 16 physiologic races of *Puccinia rubigovera tritici* (Erikss. and Henn.) Carl. prevalent in the spring wheat region were used to induce the leaf rust epidemic; a composite of 14 common physiologic races of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. was used to induce the stem rust epidemic.

Relative control and regulation of the intensity of the rust epi-

demics were obtained by means of sulfur dusting. Two methods of approach, "preventive" and "inhibitive," were employed. By the use of the preventive method, the incidence of rust was delayed by sulfur dusting until certain stages in plant development were reached; after this, artificial rust inoculations were made at weekly intervals. In the inhibitive series, rust infection was induced artificially in the early stages of plant growth; further progress of the epidemics was arrested at given periods by weekly applications of sulfur dust.

The samples used in this study were limited to plants taken from two sets of replicate test plots, eight of which represented the lightest, and eight, the severest, rust infection that prevailed. In the least severely infected plots, no artificial inoculations were made and natural rust incidence was controlled by weekly applications of sulfur dust. In the most severely infected plots, the plants were inoculated with rust at weekly intervals but received no sulfur dusting. The first samples for the biochemical determinations were cut from the Thatcher plots on July 7, those from the Marquis plots, a week later; subsequent samples were obtained at semiweekly intervals thereafter. The collection and preparation of the samples for analyses followed the methods described by Geddes and Levine (1942).

As an index of progressing maturity, the dry matter weights per thousand kernels and the moisture contents of the entire wheat tiller were determined. Five hundred air-dry kernels per sample were counted and weighed in duplicate. The moisture contents of each plant fraction were determined on samples ground in a Wiley laboratory mill (equipped with a sieve having 0.5 mm openings) by the one-hour, 130°C air-oven procedure, as described in *Cereal Laboratory Methods* (4th ed., 1941). The moisture contents of the entire tillers were computed from the original weights of the various fractions and the moisture lost on air-drying and on oven-drying.

The samples were analyzed in duplicate on different days for total phosphorus by the colorimetric procedure, outlined in *Cereal Laboratory Methods* (4th ed.). Transmission of light was determined in a Coleman Model 11 Universal Spectrophotometer at 533 mμ. The ash content of the samples was determined by ignition overnight at approximately 575°C. The phosphorus (expressed as P) and ash results were computed on the dry matter basis.

Results and Discussion

The data for kernel weights and moisture contents of the entire tillers at successive stages of maturity of Thatcher and Marquis wheat variously infected with leaf and stem rust, respectively, are presented in Table I. The percentages of phosphorus present in the three frac-

tions—grain (kernels), chaff (glumes and rachides), and straw (stems and leaves)—into which the tillers were divided, and the percentage of phosphorus in the entire tillers, are recorded in Table II. Since the weights of each fraction and the number of tillers represented were known, it was possible to calculate the total quantities of phosphorus present in each fraction and in the entire tiller. These data are given in Table III. The percentage distribution of the total phosphorus of the tiller among the three plant fractions was computed from the data of Table III and are recorded in Table IV.

Effect of Leaf and Stem Rust on Kernel Weight and Plant Transpiration. The effect of severe leaf rust infection on the weight of the developing Thatcher kernels became pronounced 3 weeks after blossoming, while the effect of stem rust on Marquis became evident a week sooner. The ultimate decrease in kernel weight of Thatcher due to leaf rust was 2.5 g per 1,000 kernels, as compared with a decrease of 6.2 g per 1,000 kernels for Marquis on account of stem rust (Table I). The increased rate of moisture loss brought about by

TABLE I

EFFECT OF MATURITY AND RUST INFECTION ON THE WEIGHT PER THOUSAND KERNELS AND THE MOISTURE CONTENT OF THE TILLERS

Days after blossoming	Successive stages of kernel development	Most lightly rusted wheat		Most severely rusted wheat	
		Weight per 1,000 kernels (dm basis)	Moisture content of aerial tiller	Weight per 1,000 kernels (dm basis)	Moisture content of aerial tiller
		g	%	g	%
Thatcher		31.7% leaf rust		89.5% leaf rust	
7	Pre-milk	3.7	70.8	4.6	71.2
10	Early milk	6.0	69.8	6.6	68.3
14	Late milk	9.1	65.2	9.7	65.1
17	Soft dough	12.5	64.0	12.5	62.6
21	Medium dough	16.1	59.0	16.5	57.2
24	Hard dough	19.9	52.2	18.1	48.8
28	Semiripe	20.1	39.6	17.7	26.2
31	Full ripe	21.2	25.8	18.7	20.8
Marquis		3.2% stem rust		48.6% stem rust	
7	Pre-milk	6.0	68.4	5.9	67.3
10	Early milk	9.9	66.0	9.7	65.6
14	Late milk	14.2	61.2	13.9	59.7
17	Soft dough	18.9	54.4	15.0	52.1
21	Medium dough	23.3	46.8	16.9	35.4
24	Hard dough	24.0	36.2	17.5	20.5
29	Near ripe	24.4	22.6	18.2	19.3

severe leaf rust infection became quite evident 24 days after blossoming and reached its peak 4 days later; in the case of stem rust, the greater moisture loss of the severely infected plants became pronounced 21 days after blossoming and reached its maximum 3 days later. Even though the average maximum stem rust infection on Marquis was only 48.6%, as compared with an average maximum of 89.5% leaf rust on Thatcher, the effects on average kernel weight and moisture content of the tiller were sharper in the case of stem rust.

Effect of Maturity on Phosphorus Content and Its Distribution. The results for the least severely infected samples of Thatcher (average 31.7% leaf rust) and of Marquis (average 3.2% stem rust) are probably the most indicative of the normal course of phosphorus metabolism and, therefore, the discussion of the effect of maturity on phosphorus content and its distribution will be based principally on the data obtained from the most lightly infected series of test plots. However, similar trends were exhibited by the results procured in the most severely rusted series.

TABLE II

EFFECT OF MATURITY AND RUST INFECTION ON THE PHOSPHORUS CONCENTRATION IN VARIOUS FRACTIONS OF THE WHEAT PLANT

Days after blossoming	Successive stages of kernel development	Percent phosphorus (P)—dry matter basis							
		Most lightly rusted wheat				Most severely rusted wheat			
		Grain	Chaff	Straw	Entire aerial tiller	Grain	Chaff	Straw	Entire aerial tiller
		%	%	%	%	%	%	%	%
Thatcher		31.7% leaf rust				89.5% leaf rust			
7	Pre-milk	0.59	0.30	0.26	0.28	0.62	0.30	0.29	0.31
10	Early milk	0.53	0.31	0.23	0.28	0.61	0.26	0.27	0.31
14	Late milk	0.51	0.25	0.22	0.28	0.56	0.23	0.23	0.31
17	Soft dough	0.54	0.23	0.18	0.28	0.54	0.24	0.21	0.30
21	Medium dough	0.49	0.24	0.16	0.26	0.55	0.21	0.17	0.31
24	Hard dough	0.51	0.22	0.12	0.28	0.53	0.24	0.15	0.29
28	Semiripe	0.56	0.24	0.10	0.28	0.54	0.25	0.13	0.28
31	Dead ripe	0.55	0.24	0.09	0.29	0.56	0.25	0.13	0.29
Marquis		3.2% stem rust				48.6% stem rust			
7	Pre-milk	0.51	0.31	0.27	0.29	0.60	0.32	0.28	0.31
10	Early milk	0.56	0.28	0.21	0.27	0.53	0.31	0.27	0.31
14	Late milk	0.53	0.29	0.19	0.28	0.55	0.28	0.24	0.31
17	Soft dough	0.55	0.26	0.17	0.29	0.56	0.31	0.19	0.31
21	Medium dough	0.53	0.24	0.09	0.25	0.56	0.33	0.18	0.31
24	Hard dough	0.57	0.23	0.09	0.28	0.57	0.26	0.15	0.29
29	Near ripe	0.56	0.20	0.08	0.27	0.59	0.14	0.12	0.27

In both Thatcher and Marquis the percentage of phosphorus in the grain showed no definite trends as maturity progressed (Table II). In the chaff the percentage of phosphorus tended to decrease slightly as the plants approached maturity. In the straw the percentage of phosphorus decreased greatly with maturity, constituting in the ripe plants only one-third of what it was at one week after flowering. In the entire tiller the concentration seemingly remained relatively constant.

When comparisons are made on the basis of total phosphorus per 100 tillers of lightly rusted plants (Table III), the phosphorus content

TABLE III

EFFECT OF MATURITY AND RUST INFECTION ON THE PHOSPHORUS CONTENT OF VARIOUS FRACTIONS OF THE WHEAT PLANT

Days after blossoming	Successive stages of kernel development	Phosphorus content (P) per 100 tillers							
		Most lightly rusted wheat				Most severely rusted wheat			
		Grain	Chaff	Straw	Entire aerial tiller	Grain	Chaff	Straw	Entire aerial tiller
		g	g	g	g	g	g	g	g
Thatcher		31.7% leaf rust				89.5% leaf rust			
7	Pre-milk	0.03	0.08	0.28	0.39	0.05	0.08	0.27	0.39
10	Early milk	0.08	0.08	0.22	0.38	0.09	0.06	0.23	0.38
14	Late milk	0.14	0.06	0.23	0.44	0.13	0.05	0.17	0.35
17	Soft dough	0.20	0.05	0.16	0.41	0.17	0.05	0.16	0.38
21	Medium dough	0.24	0.06	0.13	0.43	0.24	0.05	0.13	0.42
24	Hard dough	0.29	0.05	0.10	0.44	0.23	0.05	0.10	0.37
28	Semiripe	0.28	0.05	0.07	0.40	0.21	0.04	0.08	0.33
31	Dead ripe	0.34	0.06	0.06	0.46	0.21	0.05	0.08	0.34
Marquis		3.2% stem rust				48.6% stem rust			
7	Pre-milk	0.07	0.11	0.35	0.52	0.09	0.10	0.35	0.54
10	Early milk	0.14	0.09	0.26	0.49	0.13	0.09	0.31	0.53
14	Late milk	0.20	0.08	0.21	0.49	0.22	0.08	0.26	0.56
17	Soft dough	0.29	0.07	0.18	0.55	0.23	0.08	0.19	0.50
21	Medium dough	0.33	0.07	0.09	0.49	0.24	0.08	0.16	0.49
24	Hard dough	0.40	0.07	0.09	0.56	0.29	0.07	0.14	0.49
29	Near ripe	0.39	0.06	0.08	0.53	0.31	0.05	0.11	0.47

of the grain of both varieties increased greatly until mature, over tenfold in the case of Thatcher and about sixfold in that of Marquis. The total phosphorus content of the chaff decreased to two-thirds of its original value in Thatcher and to approximately one-half in Marquis. In the straw the total phosphorus of both varieties de-

creased to less than one-quarter of the amount present one week after flowering. In the entire tiller no clear-cut change was apparent, considering the fact that errors in determining phosphorus and dry matter are accumulated in calculating the values. The data seem to indicate that most, if not all, of the phosphorus ultimately found in the mature plant tiller is present within a week or two after blossoming and thereafter is translocated from the chaff and straw into the developing kernels.

The translocation of phosphorus into the kernels is clearly demonstrated by the changes in the percentage distribution of the total phosphorus in the three plant fractions as the plants mature (Table IV).

TABLE IV

EFFECT OF MATURITY AND RUST INFECTION ON THE DISTRIBUTION OF PHOSPHORUS IN THE WHEAT PLANT

Days after blossoming	Successive stages of kernel development	Distribution of phosphorus					
		Most lightly rusted wheat			Most severely rusted wheat		
		Grain	Chaff	Straw	Grain	Chaff	Straw
		%	%	%	%	%	%
Thatcher		31.7% leaf rust			89.5% leaf rust		
7	Pre-milk	8.7	19.5	71.8	11.9	20.1	68.0
10	Early milk	21.4	20.6	58.0	23.6	15.1	61.3
14	Late milk	32.9	14.1	53.0	36.9	13.6	49.5
17	Soft dough	48.3	12.9	38.8	44.0	13.0	43.0
21	Medium dough	56.3	12.8	30.9	57.4	11.2	31.4
24	Hard dough	67.2	11.1	21.7	61.1	12.8	26.1
28	Semiripe	70.2	12.2	17.6	63.0	13.2	23.8
31	Dead ripe	73.9	12.2	13.9	61.5	15.0	23.5
Marquis		3.2% stem rust			48.6% stem rust		
7	Pre-milk	12.8	20.6	66.6	17.3	19.1	63.6
10	Early milk	28.1	18.7	53.2	24.6	17.3	58.1
14	Late milk	41.7	16.4	41.9	39.5	14.8	45.7
17	Soft dough	53.0	13.4	33.6	46.7	16.0	37.3
21	Medium dough	68.1	13.3	18.6	49.9	16.6	33.5
24	Hard dough	71.9	12.1	16.0	58.5	13.9	27.6
29	Near ripe	74.2	10.8	15.0	66.3	9.6	24.1

In the lightly rusted Thatcher the percentage of the total phosphorus present of the entire tiller increased in the kernels from 8.7% a week after flowering to 73.9% at full maturity; in the chaff it decreased from 19.5% to 12.2%; and in the straw it was reduced from 71.8% to 13.9%. Similar translocation trends were evident in the case of lightly rusted Marquis.

It is of interest to compare these data on hard red spring wheats grown in Minnesota with the corresponding study conducted by Miller (1939) with winter wheats grown in Kansas. In contrast to our results, he found, with only one exception, that the total amounts of phosphorus in the plants reached their maximum around harvest time. However, he found very similar trends to those reported here—in the distribution of the total phosphorus in the various plant fractions, as maturity progressed. As the kernels developed, the total phosphorus in the head increased, and that in the stems and leaves decreased. Similar observations on the translocation of phosphorus during kernel development have been made by many other workers, as reviewed by Miller. In agreement with our data, he found that the percentage of phosphorus present in the stems and leaves decreased from head formation to harvest. In two of the three years in which his studies were conducted, he found that the percentage of phosphorus in the heads, in general, showed a slight decrease from the time of their formation to maturity; in the third year, the percentage of phosphorus showed a marked decrease for four weeks and then slightly increased during the final two weeks of maturity. The percentage of phosphorus in the chaff also markedly decreased from head formation to harvest. In our studies, there were no consistent trends in the percentage of phosphorus in the kernels as the grain matured, but the concentration in the glumes and rachides decreased appreciably.

The similarity in the translocation of phosphorus found in this study with that of thiamine, as reported by Geddes and Levine (1942), is very striking. However, the initial proportion of the total thiamine present in the kernels was higher, and the final percentage in the glumes and rachides was much lower than the corresponding values for phosphorus.

Effect of Rust Infection on Phosphorus Content and Its Distribution. A comparison of the results obtained for the most severely infected samples of Thatcher (average 89.5% leaf rust) and of Marquis (average 48.6% stem rust) with the data from the least severely rusted plots (Thatcher, average of 31.7% leaf rust; Marquis, average of 3.2% stem rust) provides a measure of the effect of rust on the metabolism of the wheat plant.

During the early part of the ripening period, the kernels of the severely rusted spring wheats contained a slightly higher percentage of phosphorus than the kernels of the lightly rusted wheats; these differences decreased with maturity (Table II). These results are in general agreement with those obtained by Caldwell *et al.* (1934), who found that leaf rust produced no significant changes in the percentage phosphorus present in the grain of winter wheat at various stages in

its development. Greaney *et al.* (1941) also found no differences in the percentage of phosphorus present in the mature kernels of Marquis wheat, variously affected by stem rust, in two of the three years in which their studies were carried out. In the present study the percentage of phosphorus in the chaff was not affected, in any definite manner, by severe leaf rust infection during any stage of kernel development; but severe stem rust infection tended to result, though not entirely consistently, in a slightly higher percentage of phosphorus in the chaff at the more advanced stages of maturity. However, regardless of the rust involved, the stems and leaves of the heavily infected plants contained a higher percentage of phosphorus than the lightly infected plants through the entire period of kernel development. At maturity the percentage of phosphorus in the straw of plants severely infected with either leaf or stem rust was approximately 50% greater than in that of the lightly infected plants. These results parallel closely the results obtained by Greaney *et al.* (1941) on the effect of stem rust on the percentage of phosphorus in the straw. In the entire tillers the percentage of phosphorus was slightly higher in the heavily rusted plants during the early stages of kernel development; but in the fully ripe plant the differences completely disappeared.

In contrast, the total phosphorus content of the entire tiller was markedly lowered by severe rust infection 24 days after blossoming, while its translocation into the kernel was arrested concurrently in the case of leaf rust, and a week earlier in the case of stem rust (Table III). The decrease in total phosphorus is associated with the effect of cereal rust on yield of dry matter, whereas the interference with translocation is associated with the more rapid desiccation of the severely infected plants, due to excessive transpiration.

In the early stages of kernel development an appreciably higher percentage of the total phosphorus was present in the kernels of the most severely infected Thatcher and Marquis than in those of the least severely infected plants (Table IV). Thus, a week after flowering, the most severely rusted Thatcher kernels contained 11.9%, whereas the kernels of the most lightly infected Thatcher contained only 8.7% of the total phosphorus. The corresponding values for Marquis were 17.3% and 12.8%, respectively. However, the mature kernels of the more lightly leaf rust-infected Thatcher contained 73.9% of the total phosphorus of the entire tiller, whereas, in the most severely affected plants, the ripe kernels contained only 61.5% of the total phosphorus. With Marquis, on which the average maximum stem rust infection (48.6%) was much lower than the average maximum leaf rust infection on Thatcher (89.5%), the effect of the rust on translocation was somewhat less pronounced; the mature Marquis kernels

from the most lightly infected plants contained 74.2% and those from the most severely rusted plants contained 66.3% of the total phosphorus. It is evident from the results obtained in this study that the over-all rate and extent of translocation is markedly influenced by the severity of rust infection. Thus, the difference between the final and initial percentages of the total phosphorus present in the kernels in the most lightly leaf rust-infected Thatcher was 65.2%, as compared with a difference of 49.6% for the most severely rusted Thatcher. The corresponding differences for stem rust-infected Marquis were 61.4% and 49.0%, respectively. The retarding rate of translocation in the severely rusted plants became most pronounced during the later stages of kernel development and paralleled the increased rate of transpiration associated with the severity of rust infection. The effect of transpiration on translocation seemed to occur about a week earlier in the case of stem rust, than in that of leaf rust, and continued until maturity.

Effect of Maturity on Phosphorus Content of Ash. An analysis of the data for the percentage of phosphorus in the ash showed that the severity of leaf or stem rust infection appeared to exert little material influence on the values. Accordingly, the data for variously treated samples within each variety, at corresponding maturity dates, were combined and their mean values recorded in Table V. The phosphorus content of the ash of the entire tillers of both varieties remained more or less constant as maturity progressed. However, as the plants matured, there was a relatively greater translocation of phosphorus than of the other ash constituents into the kernel. Thus, in Thatcher, the percentage of phosphorus in the kernel ash increased from 17.7% at 7 days after blossoming to 27.3% at full maturity; the percentage of phosphorus in the ash of the chaff (glumes and rachides) fell from 4.1% to 1.9%; while in the straw (stems and leaves), the phosphorus in the ash was reduced from 2.9% to 1.3% as the plant ripened. In Marquis, the preferential translocation of phosphorus into the kernel was less marked than in Thatcher, increasing from 21.1% to only 24.8% during the maturation period.

Precision of the Colorimetric Method for Total Phosphorus. The precision of the colorimetric method for phosphorus is shown by the data presented in Table VI. To determine whether the experimental errors were influenced by the magnitude of the phosphorus values, the samples were classified according to phosphorus content into three groups. Experimental errors were calculated from the duplicate determinations for the samples within each group and for the entire series. The absolute error did not increase proportionally with phos-

TABLE V

PHOSPHORUS CONTENT OF THE ASH OF VARIOUS FRACTIONS OF THE WHEAT PLANT AT SUCCESSIVE STAGES OF KERNEL DEVELOPMENT

Number of days after blossoming	Successive stages of kernel development	Percentage of phosphorus in ash of wheat plants			
		Grain	Chaff	Straw	Entire aerial tiller
		%	%	%	%
		Thatcher			
7	Pre-milk	17.7	4.1	2.9	3.5
10	Early milk	21.5	3.2	2.7	3.5
14	Late milk	22.5	2.4	2.4	3.6
17	Soft dough	24.9	2.1	2.2	3.9
21	Medium dough	26.1	1.8	1.7	3.9
24	Hard dough	26.6	1.8	1.4	3.8
28	Semiripe	26.8	1.8	1.2	3.7
31	Dead ripe	27.3	1.9	1.3	3.8
		Marquis			
7	Pre-milk	21.1	4.8	3.1	3.9
10	Early milk	22.5	4.1	2.8	3.9
14	Late milk	23.2	3.1	2.6	4.3
17	Soft dough	24.2	2.9	2.0	4.1
21	Medium dough	24.2	2.8	1.7	3.8
24	Hard dough	25.3	2.4	1.4	4.1
29	Near ripe	24.8	1.7	1.4	4.2

phorus concentration, although it is highest for the class representing samples which contained the highest percentage of phosphorus. The coefficient of variability decreased rather sharply with the increase in the mean values of the samples. The percentage errors show the precision of the method used in this study to be quite satisfactory.

TABLE VI

ERRORS OF THE COLORIMETRIC METHOD FOR DETERMINATION OF PHOSPHORUS

Class	Number of samples	Phosphorus (P)—dry matter basis			Coefficient of variability
		Class range	Class mean	Standard error per single determination	
		%	%	%	%
1	54	0.00–0.21	0.16	0.018	11.4
2	86	0.22–0.43	0.27	0.016	6.0
3	61	0.44–0.62	0.55	0.027	4.8
All	201	0.00–0.62	0.33	0.021	6.3

Summary

Total phosphorus determinations were made on three plant fractions—grain (kernels), chaff (glumes and rachides), and straw (stems and leaves)—prepared from tillers harvested during kernel filling from plots of Thatcher and Marquis wheats variously infected with leaf and stem rust, respectively. The leaf and stem rust epidemics were induced by artificial inoculations and the intensity of natural rust infection was controlled by periodic sulfur dustings. Average kernel weights and moisture contents of entire tillers were determined as indices of progressive maturity.

Average kernel weight was adversely affected by severe rust infection, particularly stem rust. Transpiration rate was accelerated to a greater extent by stem rust than by leaf rust, even though the average maximum stem rust infection was 48.6%, as compared with an average maximum of 89.5% leaf rust infection.

Percentage of phosphorus in the dry matter of the kernels remained more or less constant with progressive maturity. In the chaff (glumes and rachides) the percentage of phosphorus tended to decrease appreciably as the plants approached maturity, whereas in the straw (stems and leaves) a very consistent and most pronounced decrease occurred.

Total phosphorus in the entire tiller remained relatively constant throughout maturation, but the phosphorus was translocated from the straw and chaff into the developing kernel. The translocation of phosphorus exceeded that of the other ash constituents.

Severely rusted plants contained a somewhat higher concentration of phosphorus in the grain than the more lightly infected plants during the early stages of kernel development, but the differences were reduced as the plants approached maturity. Intensity of rust infection was without apparent influence on the percentage of phosphorus present in the chaff, but caused a definite increase in the concentration of phosphorus in the straw fraction in the final stages of maturation. The effects were similar in the case of leaf and stem rust.

Severe leaf rust infection, as that of stem rust, lowered the total phosphorus content of the entire tiller of the wheat plant and markedly interfered with its translocation into the kernels.

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RAPID DETERMINATION OF BROMATE IN FLOUR

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Various techniques have been proposed from time to time for the estimation of bromate in flour, of which the most recent is that suggested by Geddes and Lehberg (1938). Although the method described by these authors is specific for bromate in the presence of other common oxidants, it apparently is slow and difficult, because they say, "A single determination requires approximately 8 hours and one analyst can conduct up to four determinations simultaneously." They also attempted to use a technique published by Kulman (1934), in which added bromate is separated by dispersing flour in carbon tetrachloride, but reported it to be unreliable.

Although we were not aware of Kulman's work at the time, we discovered several years ago that bromate was quite commonly present when carbon tetrachloride was used to separate added phosphates from certain export flours, and that a rough estimation of the amount of such bromate by the usual volumetric procedures was entirely feasible. Some time later we worked out the details of a satisfactory procedure based on carbon tetrachloride separation. Since we have obtained entirely satisfactory results using this technique, we are herewith offering the procedure in detail.

Reagents:

(1) Carbon tetrachloride.
(2) Starch indicator solution, 1%. Suspend 1 g soluble starch in a small volume of cold water, pour into boiling water, cool and dilute to 100 ml.
(3) Sulfuric acid, 10%.
(4) Potassium iodide solution, 50%.
(5) Sodium thiosulfate, 0.01 *N*.

Procedure:

Weigh 15 g flour into a 50 ml centrifuge tube containing about 20 ml of carbon tetrachloride. Disperse the flour thoroughly by means of a spatula or stirring rod but do not allow the flour to reach the bottom of the tube. Fill the tube to within one inch of the top with additional carbon tetrachloride and centrifuge for 2 minutes at a speed sufficient to effect a clean separation between flour and liquid.

Insert a stirring rod or spatula to the point of separation, and using a circular motion again disperse the flour. Centrifuge again for 2 minutes. Repeat dispersion

with stirring rod, and centrifuge for 3 to 5 minutes or until the flour is densely packed and the carbon tetrachloride is clear.

Hold the tube in an inclined position and remove the flour by means of a small spoon or spatula. Carefully manipulate the tube until it is finally inverted and remove any flour still adhering by use of a small cotton swab, making sure that none of the solid residue at the bottom of the tube is lost. Evaporate residual carbon tetrachloride at room temperature.

Dissolve the residue in 5 to 6 ml water, add 2 ml 10% sulfuric acid, 1 ml potassium iodide solution, and 1 ml starch indicator solution and titrate with 0.01 *N* thiosulfate.

Potassium bromate, grams per cwt. = ml 0.01 *N* thiosulfate × 0.84.

In experimenting with additions of known amounts of bromate, we found that repeated resuspension of flour and centrifuging is necessary if reliable results are to be obtained. The more finely pulverized is the bromate employed, the more is it necessary to resort to multiple stage centrifuging. With these precautions, however, we have consistently obtained recoveries of 90 to 110% or better. The tabulation below gives results obtained in three different laboratories by this method on samples of flour to which known quantities of bromate had been added.

Bromate added	Added bromate recovered		
	Laboratory A	B	C
g per cwt.	%	%	%
0.75	100	—	—
1.0	99	—	—
2.0	99	—	—
2.3	102	100	94
4.5	99	98	97

This method cannot be applied to whole wheat flours which have been treated with bromate by dissolving the salt in water and applying to the whole grain. The procedure as given is not specific for bromate but will include any salt with density greater than that of carbon tetrachloride and which will oxidize an acidified iodide solution. Thus iodates, persulfates, etc., will be reported as bromate. It seems probable, however, that the technique suggested by Kolthoff and Hume (1943) could be applied to the residue and thus iodates and bromates could be quantitatively distinguished.

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A RAPID METHOD FOR THE DETERMINATION OF BROMATE IN BROMATED FLOURS

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For many years oxidizing agents such as potassium bromate, ammonium persulfate, nitrogen trichloride, and chlorine have been used as flour and dough improvers. Of these potassium bromate is one of the most commonly used and has, perhaps, been most widely studied. Probably close to 90% of all the spring wheat flour sold in Canada contains added bromate, the usual dosage being about 5 μg per g (5 ppm). A method, at once rapid and reasonably accurate, for the determination of a substance so frequently found in commercial flours is very much needed.

Geddes and Lehberg (1938) made a study of the subject, and of the methods they investigated found that the one described by Yates (1933) for the determination of bromine in blood best lent itself to the purpose in view. By an adaptation of this method, using special glass equipment designed by Binnington (1937), bromate in flour could be determined in the presence of other oxidizing agents such as periodates. The standard error of a single determination was 0.000097% (0.97 μg/g). Although four tests can be made simultaneously, a single determination by the method of Geddes and Lehberg takes about 8 hours, a fact which seriously limits the usefulness of the method for routine work.

Ford, Kent-Jones, Maiden, and Spalding (1940) also described a method for the determination of bromine in flour and provided an extensive review of the literature on the subject. Since the natural flours they examined varied in bromine content from 2.4 to 7.7 μg per g, the authors concluded that the additions of bromate usual in Great Britain, which increased the bromine content by an average of 3.6 μg per g, could not be detected by means of a bromine determination. In view of this, and because high bromine content may now be due to the use of methyl bromide as a fumigant, no reference will be made to more recent methods for determining bromine in flour.

A modification of the method outlined in *Cereal Laboratory Methods* (4th Ed., 1941) has long been used as a semi-quantitative test for bromate in flour. In brief, the method consists of dropping a mixed reagent of hydrochloric acid and potassium iodide on a smooth flour surface. The reagent is prepared just before use and is added drop by drop until the surface is uniformly wetted. Deep purple spots appear

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if bromate is present. With bromate of one uniform fineness, the number of spots per unit area is roughly proportional to the amount of the improver present in the flour. Unknowns can be compared with standards containing known dosages of the improver.

The more precise method now to be described is based on the same reaction, but the flour is first extracted and the bromate in the extracts determined by measuring the intensity of the violet color produced upon the addition of acidified potassium iodide.

Experimental

Apparatus

(1) 250 ml Erlenmeyer flasks.
(2) 50 ml centrifuge tubes.
(3) Coleman Universal Spectrophotometer, Model 11, and optically matched round cuvettes of 1.7 cm diameter.

Reagents

(1) Potassium chloride solution, 25% in 2% acetic acid.
(2) Potassium permanganate solution, 5.0 mg/ml.
(3) 5% hydrochloric acid (c.p. free from chlorine).
(4) 1% potassium iodide solution.
(5) Mixed reagent. Equal parts by volume of 5% hydrochloric acid and 1% potassium iodide mixed immediately before use.
(6) Standard potassium bromate solutions, 2.5 μg/ml and 5.0 μg/ml. These solutions are prepared as required from a solution containing 100 μg/ml which is stable.
(7) 0.5% starch solution, prepared by the following method as outlined by Platner (1944): While stirring add approximately 30 ml of a 20% sodium hydroxide solution to a suspension of 2 g of soluble starch in 300 ml of water and allow to stand for 1 hour. Neutralize with concentrated hydrochloric acid using litmus as an indicator and then add 1 ml of glacial acetic acid. Dilute to 400 ml with water. This solution can be used for an indefinite period.

Method. Weigh 2.5 g of flour into a 250 ml Erlenmeyer flask if the sample contains less than 20 μg/g of bromate and 1.25 g if it contains between 20 and 50 μg of bromate per g. Add 49 ml of 25% potassium chloride solution in 2% acetic acid. Disperse the flour thoroughly and add 1 ml of permanganate solution, shaking the flask while doing so. If 1.25 g of flour is taken, use a solution containing 2.5 mg instead of 5 mg of permanganate per ml. Allow to stand at room temperature for 15 minutes with occasional shaking. Pour the mixed suspension into centrifuge tubes and centrifuge for 10 minutes or until clear. Pipette 10-ml aliquots into 4 test tubes. To tube (1) add 6 ml of distilled water; to tube (2) 1 ml of distilled water; to tube (3) 1 ml of standard bromate solution containing 2.5 μg/ml; and to tube (4) 1 ml of standard bromate solution containing 5 μg/ml. Prepare the mixed reagent and add 5 ml to each of tubes 2, 3, and 4. Immediately add 1 ml of starch solution to all four tubes, mix the contents of each, and exactly 10 minutes after the addition of the mixed reagent, read the optical density at the absorption peak.[3] Tube (1) is the blank.

The optical density is directly proportional to the concentration of bromate. The bromate concentration in the flour is determined from the increments in optical density due to additions of the standard bromate solutions. When 2.5 g of flour is used, the concentration is given by the formula:

$$\text{Concentration of bromate, } \mu g/g = \frac{2.5 \times A \times 50}{B \times 10 \times 2.5} = \frac{5A}{B}$$

where A is the optical density of the unknown in tube (2),
B is the average of the differences in optical density between tube (3) and tube (2), and between tube (4) and tube (3).

[3] In all the determinations reported in this paper the optical density was read at 575 mμ.

Effect of Potassium Chloride Concentration. The effect of concentration of the potassium chloride used for extraction was investigated over a range of 5 to 25% using a bleached and unbleached short patent and a bleached and unbleached first clear milled from Canadian hard spring wheat. Of each flour 2.5 g was extracted with 50 ml of potassium chloride solution of the indicated concentration in 2% acetic acid and to 10 ml of each clear extract 5 μg of potassium bromate was added. No permanganate was used. The mixed reagent and starch were then added and after 10 minutes the optical density readings were taken. The results in Table I show that increasing the

TABLE I

OPTICAL DENSITY PRODUCED BY 5 μG OF POTASSIUM BROMATE IN FLOUR EXTRACTS AS INFLUENCED BY CONCENTRATION OF POTASSIUM CHLORIDE

Flour			Optical density				
			Concentration of KCl				
Grade	Ash %	Bleach	5%	10%	15%	20%	25%
Patent	0.38	None	0.064	0.076	0.133	0.147	0.165
Patent	0.38	Agene and Alsop	0.123	0.170	0.210	0.225	0.240
Clear	0.82	None	0.002	0.004	0.008	0.010	0.012
Clear	0.82	Agene and Alsop	0.000	0.005	0.005	0.014	0.014
Mean			0.047	0.064	0.089	0.099	0.108

concentration of the potassium chloride from 5 to 25% greatly increased the optical density. Because it gave the maximum readings, the 25% solution was adopted as the extractant. At all levels of potassium chloride concentration the bleached patent gave higher readings than the unbleached patent, and the unbleached patent higher readings than the clear. Before the method could be considered to possess any promise whatever, these discrepancies—apparently caused by variations in the quantity of bromate reduced [4] by the extracts of different flours while the test was being carried out—had to be eliminated.

Effect of Permanganate. To destroy the substances responsible for the loss of bromate, various oxidizing reagents were tried and of these potassium permanganate was found to be most suitable. It oxidized the flour extracts to the extent that they no longer reduced bromate while the determinations were being carried out and was itself so quickly and completely reduced that none was left to liberate iodine

[4] Though we speak of the bromate being reduced, its disappearance can be just as well explained on the theory that it is adsorbed on some constituent of the flour extract. In a subsequent paper we hope to submit evidence in support of the latter explanation.

when iodide (mixed reagent) was added. *The same results were obtained whether the permanganate was added to the flour suspension before or after the addition of the bromate.*

The same flours previously used were extracted as before except that the extractant now consisted of 49 ml of potassium chloride solution and 1 ml of a solution containing 2.5 mg of potassium permanganate. Varying concentrations of potassium chloride were again used and to a 10-ml aliquot of each clarified extract 5 μg of bromate was again added. Results of optical density measurements, taken 10 minutes after the addition of the mixed reagent, are given in Table II.

TABLE II

OPTICAL DENSITY PRODUCED BY 5 μG OF POTASSIUM BROMATE IN FLOUR EXTRACTS AS INFLUENCED BY THE CONCENTRATION OF POTASSIUM CHLORIDE, PERMANGANATE BEING ADDED TO THE EXTRACT

Flour		Optical density				
Grade	Bleach	Concentration of KCl				
		5%	10%	15%	20%	25%
Patent	None	0.114	0.175	0.190	0.220	0.235
Patent	Agene and Alsop	0.115	0.172	0.210	0.220	0.247
Clear	None	0.130	0.178	0.198	0.235	0.260
Clear	Agene and Alsop	0.120	0.158	0.200	0.230	0.255
Mean		0.120	0.171	0.200	0.226	0.249

A comparison of the results in Tables I and II shows that the addition of permanganate to the extractant had no effect on the intensity of the color developed in the bleached patent extracts, but caused pronounced increases in the readings for the unbleached patent and still greater increases with the clear. With all flours the more concentrated potassium chloride solutions again gave the higher optical densities.

The effect of permanganate may be seen more clearly from the calibration curves of optical density against bromate concentration shown in Figure 1. The plotted results were obtained as follows: To 2.5 g of the flour a mixture of 49 ml of 25% potassium chloride solution in 2% acetic acid and 1 ml of a solution containing 2.5 mg of potassium permanganate was added, followed by the specified amount of bromate as soon as the permanganate color had disappeared. The determination of optical density was then carried out according to the usual procedure. The experiment was repeated using 50 ml of potassium chloride solution without permanganate. An unbleached feed flour

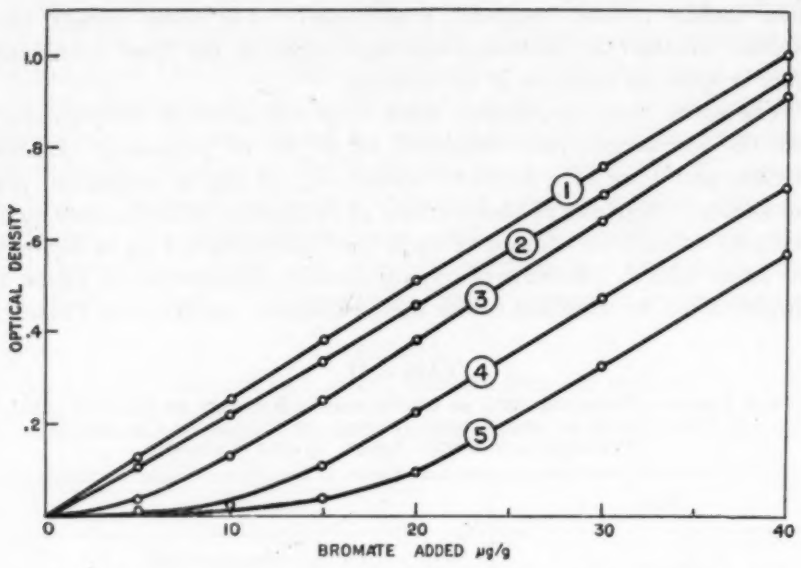

Fig. 1. Effect of increments of potassium bromate, with and without $KMnO_4$, on optical density. Curve (1) bleached and unbleached patent and clear flours with $KMnO_4$ and bleached patent without $KMnO_4$; (2) feed flour with $KMnO_4$; (3) unbleached patent, (4) bleached clear, (5) unbleached clear—without $KMnO_4$.

having an ash content of 1.40% was included in the permanganate series.

With permanganate in the extractant, the bleached and unbleached patent and clear, as well as the bleached patent without permanganate, gave five sets of results which were in such close agreement that they were taken to produce a single curve. Apparently no loss of bromate occurred while these tests were being made. Without permanganate the curve for the unbleached patent was logarithmic at lower bromate concentrations and a straight line at higher concentrations, while the curves for the clear flours became straight lines only at the highest concentrations of bromate. These three curves are interpreted to mean that, except in the straight line portions, bromate losses were taking place and that these losses were related to the bromate concentration and the degree of oxidation (grade and bleach) of the flour. By extrapolating the straight line portions of the curves to zero optical density, the maximum loss for each flour can be estimated as follows: Unbleached patent 4 to 5 µg per g of flour, bleached clear 11 µg, and unbleached clear 16 µg.

Feed flour with permanganate gave a straight line curve which, however, upon extrapolation, did not pass through the origin, the indicated loss of bromate being 1 µg per g of flour. It was a very low-grade product and was used, not as an example of a bromated

flour of commerce but in order to include flours at both extremes of the grade scale.

The effect on the optical density of using different quantities of permanganate was studied with results shown in the curves which make up part of Figure 2.

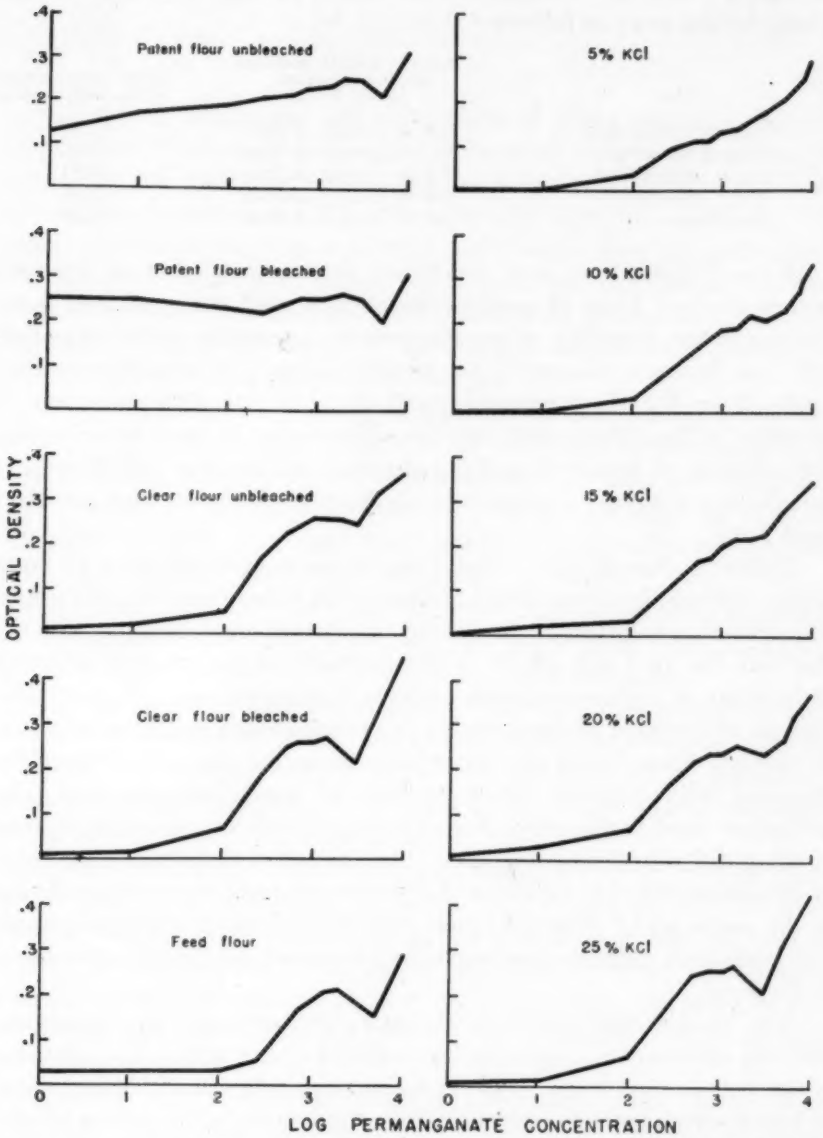

Fig. 2. Effect on optical density of varying permanganate concentration from 1 μg to 10,000 μg per g of flour. (Left) Using different grades of flour. (Right) Using different concentrations of KCl with the same flour (bleached clear).

Each flour was suspended in the 25% potassium chloride solution, permanganate was added, and this was followed by 10 μg of bromate per g of flour, after the permanganate color had disappeared. The regular procedure was then followed. The curves differ rather widely, but they show that for each flour there is a range of permanganate additions which produce almost constant optical density readings. These ranges were as follows:

Flour	Range of $KMnO_4$ additions producing uniform optical density	Mean optical density within $KMnO_4$ range
Patent unbleached	0.5 to 3 mg/g of flour	0.232
Patent bleached	0 to 3 mg/g of flour	0.246
Clear unbleached	0.5 to 3 mg/g of flour	0.251
Clear bleached	0.5 to 2 mg/g of flour	0.256
Feed flour	1.0 to 3 mg/g of flour	0.200

From Figure 1, it was concluded that a small loss of bromate occurred when 1 mg of permanganate was used per g of feed flour. Since a larger quantity of permanganate apparently failed to reduce this loss, bromate cannot be accurately determined in such very low-grade flour by the proposed method. For the determination of bromate in the short patent and clear flours used in these experiments, the addition of from 0.5 to 2 mg of permanganate per g of flour gave satisfactory results. In our own application of the method we have used 2 mg.

Table II showed that, using 1 mg of permanganate per g of flour, higher optical densities were obtained with more concentrated potassium chloride solutions. It seemed of interest to determine whether this was due to a salt effect on the intensity of the color or to losses of bromate at lower potassium chloride concentrations. In the latter case an adjustment of the quantity of permanganate might be expected to prevent these losses and thus bring into line the optical densities obtained with different concentrations of potassium chloride. The procedure used in the preceding experiment was adopted, but instead of using different flours, the same flour (bleached clear) was suspended in potassium chloride solutions of different strength made up as always in 2% acetic acid. The combined effect of variations in permanganate and potassium chloride concentrations is shown by curves reproduced in Figure 2.

The results indicated that the more concentrated the potassium chloride solution the less reducing material it extracts. The plateaus in the curves for 20 and 25% potassium chloride are wider and occur at lower permanganate concentrations than those in the curves for the 10 and 15% solutions. The 5% curve showed no plateau, the optical density increasing progressively with increasing amounts of permanga-

nate. Not all of these features are well shown by the separate log curves, but the essential data are as follows:

KCl concentration	Range of $KMnO_4$ additions producing uniform optical density	Mean optical density within $KMnO_4$ range
25%	0.5 to 2 mg/g of flour	0.256
20%	0.75 to 3 mg/g of flour	0.246
15%	1.5 to 3 mg/g of flour	0.223
10%	2 to 5 mg/g of flour	0.220
5%	—	—

In strong potassium chloride extracts little bromate-reducing material is present and this is satisfied by small additions of permanganate. In the 25% extract 0.5 mg of permanganate was sufficient, but amounts up to 2 mg did not materially alter the readings. The extra permanganate was evidently reduced to produce compounds which did not react with iodide. Oxidation with still larger amounts of permanganate, however, led to the formation of products capable of liberating iodine from iodide. (No explanation can be suggested for the dips in the curves shown in Figure 2.)

When much reducing material is present in solution, as is the case with the 5% potassium chloride extract, relatively large quantities of permanganate must be added to prevent loss of bromate, if, indeed, it is completely prevented. Probably before this stage is reached, oxidation products that react with iodide have already been formed, with the result that there is no plateau in the curve.

The test thus depends on the fact that 25% potassium chloride in 2% acetic acid extracts bromate without, at the same time, taking up relatively large amounts of strongly reducing substances.

The preceding experiment shows that the low optical density results in Table II were due to the use of insufficient permanganate or, in the 5% potassium chloride extract, to the presence of interfering material. That it was not due to a salt effect on the color is confirmed by the results of the next experiment.

Extracts of bleached and unbleached clear were made with different concentrations of potassium chloride, and to one aliquot of each extract, bromate equivalent to 10 μg per g of flour was added and to another the equivalent of 20 μg per g. The differences in optical density readings between the pairs of aliquots are reported in Table III.

Variations in the potassium chloride concentration from 10 to 25% had little if any effect on the optical density.

Effect of Time and Temperature of Extraction. The results were unaffected by varying the time of extraction from 15 to 60 minutes or by varying the temperature of a 15-minute extraction from 0° to 50°C. Bromate losses during extraction in the absence of permanganate

TABLE III

INCREASE IN OPTICAL DENSITY OF EXTRACTS CONTAINING 20 μG/G OF POTASSIUM BROMATE OVER EXTRACTS CONTAINING 10 μG/G OF BROMATE AS INFLUENCED BY CONCENTRATION OF POTASSIUM CHLORIDE

Flour and treatment	Optical density				
	Concentration of KCl				
	5%	10%	15%	20%	25%
Bleached clear	0.210	0.242	0.245	0.250	0.245
Unbleached clear	0.220	0.242	0.247	0.250	0.250

increased at 70°C, and when the temperature was raised to 100° all the bromate was reduced.

Stability of Color. When the optical density of a blank, prepared by adding mixed reagent to an unbromated flour extract in 25% potassium chloride, was deducted from the optical density of the same extract containing bromate, it was found that the color intensity due to the bromate increased for 6 or 7 minutes after the addition of iodide and then remained stationary. The optical densities of the bromated extract and the blank continued, however, to show an upward drift. In the interval from 10 to 20 minutes after the mixed reagent was added, the mean increase in 16 experiments was 0.015, equivalent to 0.6 μg per g of bromate. This increase took place whether permanganate was added or not and was due to the slow liberation of iodine by other reagents, by substances other than bromate in the flour extract, or by dissolved oxygen. Because of this change in color with time, readings must be made exactly 10 minutes after the mixed reagent is added.

Characteristics of the Starch-Iodine Color. In our analyses of commercial flours, the starch-iodine solutions showed a maximum light absorption at a wave length of approximately 575 mμ and we have used that wave length consistently. Soluble starch is added to the extracts in order to ensure an excess, especially when dealing with heavily bromated flour. When iodine reacted with the soluble starch alone, the maximum light absorption was found to be at 600 mμ. In spite of the addition of soluble starch, the absorption characteristics are always largely determined by the nature of the reacting carbohydrates extracted from the flour. Fortunately, small variations in the absorption maximum can be safely ignored since the absorption peak is broadly rounded and because an internal standard is used.

The potassium chloride extract of a 15-year-old flour (with the usual amount of added soluble starch) showed a maximum absorption

at 420 mμ, but some factor other than age alone must have been responsible for this low reading, for on testing a sample 39 years old, its peak was found to be at 550 mμ. It is not known to what extent the absorption characteristics of the starch-iodine solution are influenced by the type and grade of wheat from which flours are produced or by other factors such as flour storage conditions. Certain low-grade flour streams give solutions having a maximum absorption at lower wave lengths, i.e., they are more reddish in color than usual. In case of doubt the absorption characteristics should be investigated, as may be quickly and easily done by means of a spectrophotometer.

Interference by Other Improvers. Of the following oxidizing agents which were added to flour at a concentration of 100 μg per g, potassium persulfate, ammonium persulfate, potassium iodate, potassium periodate, potassium chlorate, and potassium perchlorate, only the iodate and periodate reacted with the mixed reagent. Consequently, bromate cannot be estimated by the rapid method in the presence of either of these two salts. Ordinary dosages of nitrogen peroxide, nitrogen trichloride, and chlorine do not interfere with the test. Heavily chlorinated flours may react with the mixed reagent, but such flours are seldom, if ever, treated with bromate.

Precision of the Rapid Method. Twenty bromated flours were analyzed in duplicate. The mean of all the determinations was 13.17 μg per g and the mean difference between duplicates, which were run on different days, 1.01 μg per g. The standard error of a single determination was 0.97 μg per g.

To find what part of the error was associated with the steps involved in the development and measurement of the color, two aliquots of each of 16 extracts of bromated flours were taken for the measurement of optical density. The standard error was found to be 0.41 μg per g, or nearly half the total error.

Summary

Potassium bromate in flour may be quickly determined by extracting with 25% potassium chloride in 2% acetic acid, allowing the extracted bromate to react with acid potassium iodide, and measuring the optical density of the amylose-iodine solution at the maximum light absorption.

The method depends upon the fact that concentrated potassium chloride solution extracts only small quantities of interfering substances. Sufficient permanganate is added to the extractant to destroy these substances without the formation of oxidation products which react with iodide.

Below 50°C the temperature and time of extraction are not critical factors.

Bromates in commercial flours have been determined by measuring optical densities at a fixed wave length of 575 mμ, but wider experience may show the necessity for varying this wave length according to the absorption characteristics of the starch-iodine solution.

Spring wheat flours ranging in grade from short patents to first clears have been analyzed by the method. It cannot be applied to very low-grade flours since they yield extracts which react with bromate while the test is in progress.

Bromate cannot be determined by the proposed method in the presence of iodate or periodate. Other improvers, when used in the dosages commonly applied to bread flour, do not interfere.

The standard error of a single determination is 0.97 μg per g of which approximately half is due to errors in sampling and extraction.

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THE DISTRIBUTION OF THIAMINE IN WHEAT SEEDLINGS AT DIFFERENT STAGES OF GERMINATION IN THE DARK [1]

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Inquiries arising from the efforts of nutritionists to increase the vitamin content of the diet have led to a suggestion that the use of germinated cereals should be encouraged (Nutrition Reviews, 1943). Burkholder and McVeigh (1942) reported significant increases in

[1] Paper No. 2251, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[3] Chief Chemist, Purity Flour Mills Limited, Winnipeg, Manitoba.
[4] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

riboflavin, niacin, biotin, and pyridoxine during germination of cereals but found no change in total thiamine, although the concentration of this vitamin in green seedling leaves was high compared with that in the original seed. A later summary presented by Burkholder (1943) pointed to the conclusion that a slight increase in thiamine, accompanied by very marked increases in riboflavin and niacin, occurred when seeds were germinated in a greenhouse for five or six days. On the other hand, McVeigh (1944) found no significant change in the thiamine content of oat seedlings which were allowed to grow in the dark for five days. The leaf showed absolute increases; the coleoptile little or no change. Davis, Laufer, and Saletan (1943) reported that malted barley and wheat were much higher in riboflavin, a little higher in niacin and pantothenic acid, and a little lower in thiamine than the unmalted samples. From these reports it appears that when cereals are allowed to germinate in the light, small increases take place in the thiamine content of the plant as a whole, but in the dark there is no increase in total thiamine, though some transfer from seed to leaf occurs. The purpose of the present study was to investigate the content and distribution of thiamine in the organs of the wheat seedling at successive stages of germination in the dark.

Experimental

A sample of Marquis wheat was steeped to 44% moisture at 10°C (50°F) and transferred to a dark germination chamber, described by Meredith, Hlynka, and Sallans (1944), that was maintained at 11.7°C (53°F) and 100% relative humidity.

Samples were removed at intervals over an 18-day period and a selected number of seedlings immediately dissected into kernel and sprout sections. The kernel was divided transversely into approximately equal brush and germ portions. At the end of the fourth day the sprout was large enough to divide into upper and lower fractions. The upper fraction comprised the coleoptile, stem, and any foliage leaves that had formed; the lower fraction consisted of the scutellar node and the rootlets. To locate the thiamine in the sprout in greater detail, the lower fraction of the samples germinated for 18 days was further dissected into two portions, one consisting of the root system and the other the scutellar node. The various fractions were air-dried, weighed, and analyzed for thiamine by the regular thiochrome method outlined in *Cereal Laboratory Methods* (4th ed., 1941), except that the sodium hydroxide and potassium ferricyanide solutions were combined before use. Sample and extractant were thoroughly mixed by means of a Waring Blendor. The determinations were made both with and

without takadiastase and all the results are reported on an air-dry basis (approximately 10% moisture).

Results and Discussion

The distribution of the air-dry weight of the seedling between the two kernel fractions and the sprout, together with the values (using diastase) for thiamine concentration, total thiamine per 100 seedlings, and the distribution of the total thiamine of the seedling in the three fractions at different periods of growth are shown graphically in Figure 1. The corresponding data for the upper and lower fractions of the sprout in comparison with the entire sprout are represented in Figure 2.

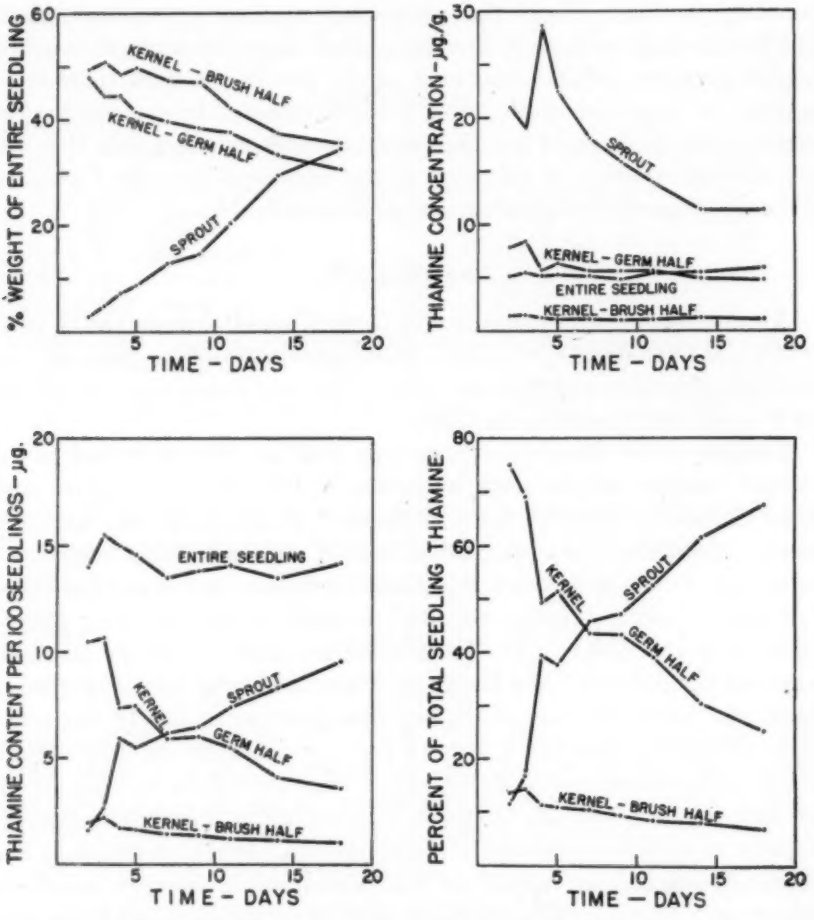

Fig. 1. Weight distribution and the concentration, content, and distribution of thiamine in different fractions of the wheat seedling at successive stages of germination in the dark.

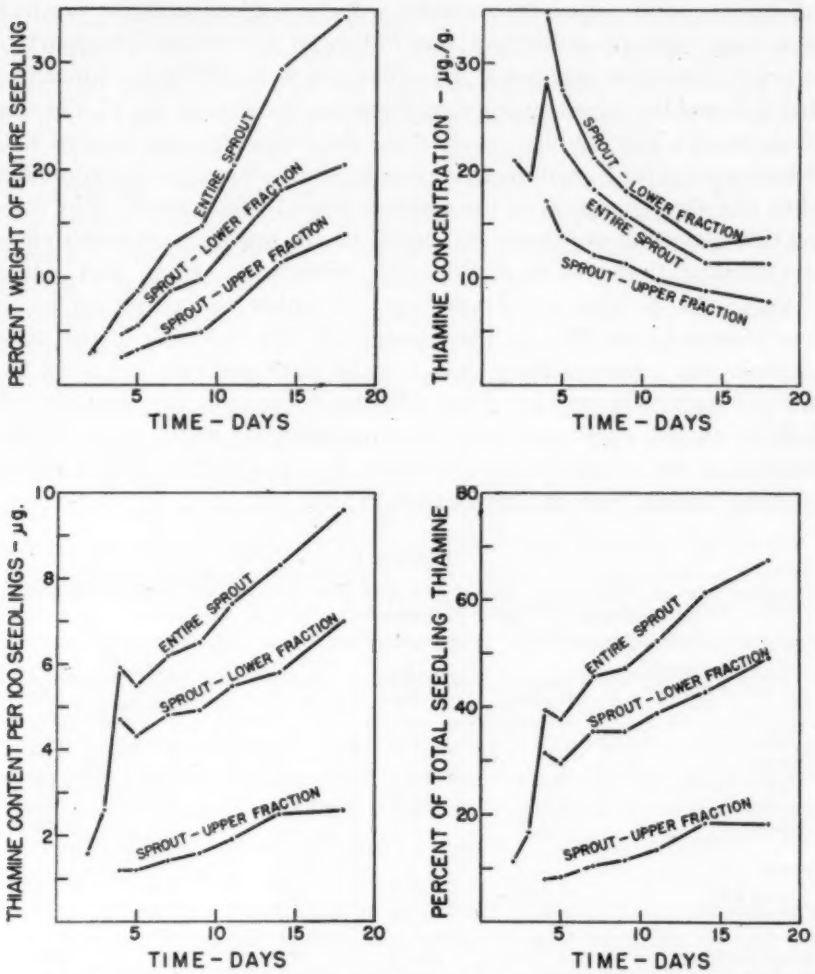

Fig. 2. Weight distribution and the concentration, content, and distribution of thiamine in the entire wheat sprout and its upper and lower fractions at successive stages of germination in the dark. The upper fraction of the sprout comprised the coleoptile, stem, and any foliage leaves that had formed; the lower fraction consisted of the rootlets and scutellar node.

The air-dry weight of the sprout relative to that of the entire seedling increased regularly from approximately 3% at 2 days germination to about 34% at 18 days germination.

The thiamine concentrations in the brush and germ halves of the kernel at two days germination were 1.4 and 7.9 µg/g air-dry weight respectively as contrasted with 20.9 µg/g in the sprout. After an apparent slight decrease between the second and the fourth day, the thiamine concentration of the brush half of the kernel remained relatively constant at approximately 1.1 µg/g during the remainder of the 18-day period. In the germ half, however, there was a decrease to

4.8 μg/g as germination progressed—a decrease that probably occurred at a more uniform rate than was indicated by values influenced by errors in dissection and assay. In the sprout, the thiamine concentration fell rapidly (after an apparent increase at 4 days) to 11.3 μg/g at 18 days. In the seedling as a whole the concentration showed little change except for a small increase during the later stages of germination when the air-dry weight of the seedling slightly decreased. The lower fraction (rootlets and scutellar node) of the sprout was much richer in thiamine than the upper fraction (coleoptile, stem, and foliage leaves). At 4 days germination the thiamine concentration in the lower fraction was 34.9 μg/g as compared with 17.1 μg/g in the upper fraction; the corresponding values at 18 days germination were 13.8 and 7.7 μg/g (Figure 2). When the lower fraction was further subdivided on the eighteenth day into rootlets and nodal tissue, it was found that the thiamine concentration in the scutellar node was considerably higher than in the rootlets (Table I).

TABLE I

DISTRIBUTION OF THIAMINE, INCLUDING COCARBOXYLASE, IN WHEAT SEEDLINGS AFTER 18 DAYS GERMINATION IN THE DARK

Fraction of plant	Weight per 100 plants[1]	Distribution of weight	Thiamine[1] concentration	Thiamine content per 100 seedlings	Distribution of thiamine
	g	%	μg/g	μg	%
Sprout					
Upper fraction [2]	0.344	13.9	7.7	2.6	18.3
Scutellar node	0.268	10.8	17.5	4.7	33.1
Rootlets	0.238	9.6	9.6	2.3	16.2
Kernel					
Germ half	0.749	30.3	4.8	3.6	25.4
Brush half	0.871	35.4	1.1	1.0	7.0
Entire seedling	2.470	100.0	5.8	14.2	100.0

[1] Air-dry basis (approximately 10% moisture).
[2] Coleoptile, stem, and foliage leaves.

Because of changes in the weights of the various fractions, the concentration data fail to indicate the changes in the thiamine content of the seedling and its fractions as germination progresses. These can be seen by computing the total thiamine values per hundred seedlings or seedling fractions. No significant change occurred in the quantity of thiamine present in the entire seedling, but the sprout increased in thiamine at the apparent expense of the brush and germ halves of the kernel, particularly the latter. From the second to the eighteenth day the percentage of the total thiamine of the seedling present in the sprout increased from 11% to 68%, while in the germ

end of the kernel it decreased from 75% to 25% and in the brush end from 14% to 7%. The decrease in the total thiamine present in the brush end began at the fourth day when the germ end had already lost about one-third of the thiamine it had contained on the second day. The total thiamine content of the upper and lower fractions of the sprout increased at approximately the same relative rate (Figure 2). After 18 days roughly half the thiamine in the sprout was in the nodal tissue, the remainder being about equally divided between the rootlets and the coleoptile-stem-leaf fraction (Table I). The increase in the total thiamine content of the sprout took place relatively faster than the increase in air-dry weight, especially during the early stages of growth. Thus on the fifth day the sprout contained about 9% of the air-dry weight and 38% of the thiamine in the entire seedling, and at the eighteenth day, 34% of the weight and 68% of the thiamine.

The increase in the proportion of the total thiamine of the seedling which is found in the sprout as germination proceeds does not necessarily prove that thiamine is translocated from the kernel (particularly the germ half) during germination. The data can also be explained by assuming that the vitamin is utilized and destroyed in the kernel, whereas it is synthesized in the sprout. However, since the total thiamine in the entire seedling remains virtually constant throughout the whole period of germination, this interpretation implies an exact balance between the rate of destruction and of synthesis. It therefore seems more logical to interpret the results as indicative of the translocation of thiamine from the kernel to the sprout where it is required for the active respiratory processes occurring there. McVeigh (1944), however, in a similar study of the distribution of B vitamins in oat seedlings considers that vitamin synthesis occurs in the sprout (particularly the leaves). She states that as the seedling grows, the vitamins are constantly being used so that "the assays do not indicate the total amounts of vitamins produced within the seedling but rather the excess of production over the amounts used." In her studies with oat seedlings grown in the dark, she found significant increases in total riboflavin and niacin but little or no change in total thiamine.

Results of thiamine determinations without the use of takadiastase are reported in Tables II and III as free thiamine; the differences between these values and those obtained when enzyme was used are reported as bound thiamine. Since they are influenced by errors of both determinations, the bound thiamine values are not very reliable, but they suffice to show that germinating wheat contains very little cocarboxylase and that most of it is in the sprout. From the results reported by Hoffer, Alcock, and Geddes (1943) with respect to the

cocarboxylase content of commercial wheat germ and other mill products, it appears that little, if any, synthesis of this substance occurs during germination, or that destruction balances synthesis.

TABLE II

FREE AND BOUND THIAMINE CONCENTRATION OF FRACTIONS OF WHEAT SEEDLINGS AT SUCCESSIVE STAGES OF GERMINATION IN THE DARK

Germination period	Free thiamine concentration[1]					Bound thiamine concentration[1]				
	Kernel		Sprout		Entire seedling	Kernel		Sprout		Entire seedling
	Brush half	Germ half	Lower fraction[2]	Upper fraction[3]		Brush half	Germ half	Lower fraction[2]	Upper fraction[3]	
Days	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4	1.0	5.5	30.2	12.9	4.8	0.2	0.2	4.7	4.2	0.3
5	1.0	5.8	23.4	11.8	4.6	0.1	0.5	4.1	2.0	0.6
7	1.0	5.2	17.6	11.3	4.5	0.1	0.4	3.5	0.8	0.6
9	1.1	5.7	16.7	11.6	4.9	0.0	0.0	1.3	0.0	0.0
11	1.0	5.5	13.3	9.9	5.0	0.1	0.1	2.8	0.0	0.4
14	1.1	4.7	12.4	7.7	5.1	0.1	0.2	0.6	1.1	0.3
18	1.1	4.9	12.7	6.6	5.4	0.0	0.0	1.1	1.1	0.4

[1] Air-dry basis (approximately 10% moisture).
[2] Rootlets and scutellar node.
[3] Coleoptile, stem, and any foliage leaves that have formed.

TABLE III

DISTRIBUTION OF FREE AND BOUND THIAMINE IN WHEAT SEEDLINGS AFTER 18 DAYS OF GERMINATION IN THE DARK

Fraction of plant	Thiamine[1] concentration		Thiamine content per 100 plants		Distribution of thiamine	
	Free	Bound	Free	Bound	Free	Bound
	μg/g	μg/g	μg	μg	%	%
Sprout						
Upper fraction [2]	6.6	1.1	2.3	0.3	17.3	33.3
Scutellar node	16.9	0.6	4.5	0.2	33.8	22.2
Rootlets	8.1	1.5	1.9	0.4	14.3	44.5
Kernel						
Germ half	4.9	0.0	3.6	0.0	27.1	0.0
Brush half	1.1	0.0	1.0	0.0	7.5	0.0
Entire seedling	5.4	0.4	13.3	0.9	100.0	100.0

[1] Air-dry basis (approximately 10% moisture).
[2] Coleoptile, stem, and any foliage leaves that have formed.

The results obtained in this study, like those reported by others, show that the thiamine content of wheat cannot be increased by germination in the dark. Indeed, if the sprouts are removed during subsequent processing, as is the case when barley is malted, appreciable losses of thiamine will occur. But if the total thiamine content of germinating wheat does not increase, neither does it decrease. Either

thiamine was not destroyed as a result of its participation in the physiological activities associated with germination and seedling growth, or the rates of destruction and synthesis were equal.

A single experiment showed that when the 18-day seedlings were exposed to the light, synthesis of thiamine occurred very rapidly. The synthesized thiamine was found distributed over the kernel, and the various fractions of the sprout.

Summary

Total thiamine content of wheat seedlings grown in the dark was essentially constant over an 18-day germination period at 11.7°C. From the second to the eighteenth day, the percentage of the total thiamine present in the sprout increased from 11% to 68%, while in the germ end of the kernel it decreased from 75% to 25% and in the brush end from 14% to 7%. These results indicate that thiamine is translocated from the kernel to the developing sprout. Preferential translocation of thiamine relative to total air-dry material was most pronounced during the early stages of growth.

Thiamine concentrations of 1.4, 7.9, and 20.9 μg/g air-dry material respectively in the brush half, germ half, and sprout at 2 days germination fell to 1.1, 4.8, and 11.3 μg/g respectively at 18 days germination.

Thiamine concentration in the fraction of the sprout comprising the rootlets and scutellar node was approximately twice that of the remaining structures (coleoptile, stem, and leaves). Further dissection of the sprout after 18 days germination showed the scutellar node to have a much higher thiamine concentration than any other part of the seedling.

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FURTHER STUDIES ON THE MECHANISM OF THE ACTION OF OXIDATION AND REDUCTION ON FLOUR

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The mechanism of the action of oxidation and reduction on flour has long been the subject of extensive research and considerable controversy. The theory that sulfhydryl compounds affect dough by means of activation of naturally occurring proteolytic enzymes in the flour has been forwarded by Jørgensen (1935, 1938) and by Balls and Hale (1936, 1938). On the other hand, this point of view has been opposed by groups who interpret the action of sulfhydryl as affecting the gluten structure. The investigation of Baker, Parker, and Mize (1942, 1943) on the role of pentosans in affecting dough quality offers a possible mechanism of action of oxidizing and reducing agents that excludes both the proteolytic theory and the view of direct chemical protein alteration. Actually there is no conclusive proof which theory is correct since all of the work reported gives data which are at best only suggestive and the interpretation varies widely.

The foundation on which the proteolytic theory has been developed began with the conclusion of Jørgensen (1935) that flour contains "powerful but latent" proteolytic enzymes of the papain type. Jørgensen (1938) supported this point of view by experiments demonstrating a decrease in the liberation of water-soluble nitrogen in flour extracts containing added bromate. Balls and Hale (1938) used their demonstration of the papain nature of the proteolytic enzyme in wheat bran as a prop to the theory; and in a later paper (1940) they found a sulfhydryl-containing substance in the petroleum ether extract of flour which they stated might serve as a natural protease activator that could, in this capacity, modify the gluten. Later, Elion (1943) attempted to lend credence to the proteolytic theory by experiments showing that addition of glutathione or a papain preparation to dough had a softening effect, while dough made from heated flour was softened only by the papain preparation. Since the latter must have contained sulfhydryl compounds, there is no conclusive evidence that the protease in the preparation played a role. Even in the case of the heated flour, the differences observed may have been due to the quantitative difference in sulfhydryl applied as glutathione and in the papain preparation. There can be no doubt concerning the fact that addition of sulfhydryl compounds softens dough, but this fact alone constitutes no proof that the effect is due to proteolysis rather than some other mechanism.

On the basis of a variety of hypotheses, other workers such as Bungenberg de Jong (1938), Ford and Maiden (1938), Read and Haas (1939), and Swanson and Dines (1939) opposed the proteolytic theory. Opposition was also presented by Sullivan, Howe, Schmalz, and Astleford (1940) whose work indicated that changes in the sulfur linkages in the gluten proteins could be responsible for the effects of oxidizing and reducing substances on dough. This point of view was strengthened by the work of Olcott, Sapirstein, and Blish (1943) indicating that the primary effect of reducing agents on gluten and dough is a chemical one upon the proteins and only secondarily that of enzyme activation. Sandstedt and Fortmann (1943) state that the action of reasonable quantities of reducing agents is reversible by oxidation, but the action of papain is not. Because the action of naturally occurring reducing agents in flour may be reversed by subsequent oxidation, these authors give this as evidence that the reduced character of doughs and bread made from unoxidized flour is not due to proteolysis.

The reversibility of oxidation and reduction of the sulfhydryl groups as they exist in gluten, in glutathione, or as a constituent part of any proteolytic enzyme, needs to be interpreted with a great deal of caution.

In their work on the effects of cysteine, glutathione, and papain on gluten demonstrated by gluten recovery and mixogram patterns, Swanson and Andrews (1945) found that, unlike reducing agents, papain affected the amount of gluten recovered and this amount was greatly decreased by increasing the amount of papain added or by lengthening the rest periods on the dough mixer. These authors could detect no activation of the latent proteases present in flour by the action of yeast-water, cysteine, or glutathione.

The purpose of this paper is to test the proteolytic theory by studying the effects of flour protease inhibitors on farinograph curves in order to determine whether the effects observed are compatible with those that would be predicted on the basis of the theory. Sodium fluoride and hexylresorcinol were employed for this purpose since Howe and Glick (1945) showed them to be potent inhibitors of wheat protease.

Experimental

An untreated patent flour milled from a Northwest spring wheat was chosen for this study. A water extract of wheat germ, prepared and measured for proteolytic activity as described previously by Howe and Glick (1945), was used as a rich source of protease. Farinograph curves were obtained in the usual fashion, with the water replaced by each of the following: a $M/7$ acetate buffer of pH 5.0, an acetate buffer-germ extract mixture (1 : 1), and the buffer-extract mixture containing 2.5 mg hexylresorcinol or 4.0 mg sodium fluoride per ml of germ extract.

In addition, the inhibitors were used in the buffer solution without germ extract; and a germ extract was also employed without buffer in which the enzyme was inactivated, as shown by titration, by heating at 80°C for 35 minutes. Howe and Glick (1945) found that a pH of 5.0 was optimal for the protease activity of both germ and patent flour extracts; and the concentrations of hexylresorcinol and sodium fluoride used effected 60% and 57% inhibitions, respectively, of the protease in the germ extract. A uniform absorption of 62.5% was used on Curves 1, 2, 3, and 4 (Figure 1) and 69% on Curves 5, 6, 7, 8, and 9 to eliminate

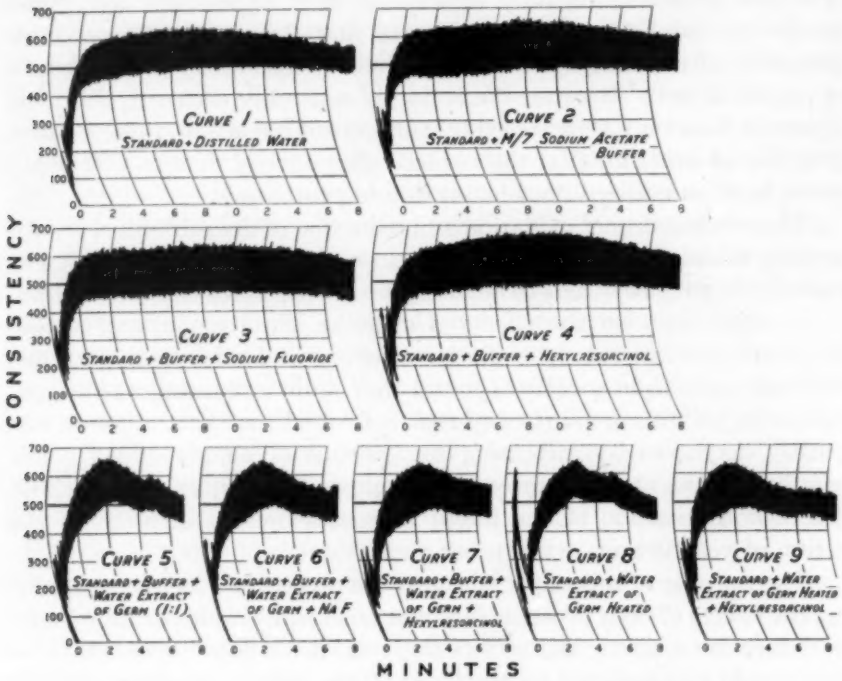

Fig. 1. Farinograph curves showing the effect of buffered protease inhibitors with and without germ extract.

any possibility of variation due to changing amounts of buffer. The increased absorption is necessary when using the water extract of germ because of the presence of solids in the extract.

Discussion

From Curves 1 and 2 (Figure 1), it is apparent that substitution of water by the particular buffer used, with or without the inhibitors, has little influence on the farinograph curve other than causing a wider amplitude. The water extract of germ had a deleterious effect on the curve. This confirms similar results demonstrated by Sullivan, Howe,

and Schmalz (1936, 1937). The addition of the inhibitors to the germ extract did not affect significantly the profile of the farinograph curve. The proponents of the proteolytic theory might claim that the damaging effect of heated germ shown by Sullivan *et al.* (1937) is due to the sulfhydryl content of the extract which could activate the flour protease. However, in Curve 9 it may be seen that, in the presence of hexylresorcinol, the slight improvement produced is about the same as the improvement made by hexylresorcinol on unheated germ extract. This small effect of the hexylresorcinol, therefore, does not seem to be due to its enzyme inhibiting property but rather to some other effect such as that on surface activity.

From the foregoing results it is clear that proteolysis does not seem to explain the effect of the water extract of germ on dough quality. These experiments suggest that the basis of the action of oxidizing and reducing agents must be sought for in some other mechanism.

Summary

The presence of sufficient hexylresorcinol or sodium fluoride to reduce drastically enzyme activity had almost no effect on dough quality as measured by farinograph curves. These findings very strongly indicate that the proteolytic theory of the effect of oxidizing and reducing agents on dough quality is not valid.

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ANALYSES OF DOUBLE-CROSS HYBRID CORN VARIETIES PRODUCED ON FARMS

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Corn is the grain crop having the largest planted acreage, the largest bushel production, and the greatest farm value in the United States. Nearly 100 million acres are planted each year, yielding a long-term average of approximately 2½ billion bushels. During the last decade, however, the use of hybrid varieties, with their drought-resistant characteristics and higher-yielding capacities, has raised the average production to about 3 billion bushels. This trend toward the use of hybrids has rapidly and steadily increased until, in 1945, 64% of the total United States acreage and 88% of the Corn Belt acreage was planted with hybrid seed.

Approximately four-fifths of the corn produced in this country is utilized for feed, food, and seed purposes on the farm where produced, while the remainder, or about 500 million bushels, moves into commercial channels. Since the utility of corn either on or off the farm is influenced by its chemical composition, information on the composition is desirable, especially for industrial purposes.

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Essentially all the corn used in industry comes from the Corn Belt, and the general use of hybrid seed in this area focuses attention on the composition of the hybrid varieties now in production. In order to determine the variations which may occur among varieties, samples for analysis were collected from farm fields planted with hybrid corn seed of known variety and source.

Comparatively little information has been published previously concerning the chemical composition of specific corn varieties.

Morrison (1936) reported the average of recent analyses of corn grain and stated that in the development of the present high-yielding varieties of corn there has been an appreciable lowering of the oil content, a slight lowering of the protein, and slight increases in starch and fiber. His data no doubt include both hybrid and open-pollinated varieties.

Ionescu and others (1939) in Roumania found that the starch and oil contents were higher and protein content lower in hybrids than in corresponding pure strains. They found an inverse relationship between protein and starch contents and a direct relationship between the amount of protein and the amount of oil.

Woodworth (1942) reported that selection for high-low chemical composition in a variety of white corn for 41 generations (1896 to 1937) has resulted in low and high strains for oil and protein. The low strains have not been altered much during the last 20 years, but the high strains have shown a consistent increase. During the period from 1934 to 1937, the high protein strain averaged 20.43% protein and the low strain 8.10% protein. During the same period the high oil strain averaged 12.02% oil and the low strain 1.62% oil.

Doty, Bergdoll, and Miles (1943) found that there were no significant differences in the chemical composition of more than 40 commercial hybrids and open-pollinated varieties of dent corn. The composition of yellow varieties tested was practically the same as that of white varieties grown under the same conditions. They determined ash, protein, oil, fiber, and starch.

Materials and Methods

In this paper composition data are presented on 159 samples of hybrid corn of known variety obtained from farm fields in 1941. One hundred and two of these were received at the laboratory as ear corn. For purposes of comparison, similar data also are presented for five hybrid varieties grown at each of three experiment stations.

The collected samples, after drying to a moisture content of about 15% at ordinary room temperature and humidity conditions, were stored at a temperature of 38° to 40°F and a relative humidity of 55 to

60%. Before storage the ear corn samples were shelled and the proportion of cobs to shelled corn determined.

Weight-per-bushel determinations of the shelled corn samples were made following the method prescribed by the Official Grain Standards of the United States. The weight per 1,000 kernels was determined on whole, sound kernels.

All foreign material and damaged kernels were removed from the shelled corn samples before grinding. They were very finely ground in a Bauer mill and analyzed by methods differing from those of the Association of Official Agricultural Chemists only in the following details. Moisture was determined by heating at 130° C for 2 hours, and ash by heating at 550°C for 4 hours. Boric acid was used to receive the ammonia in the Kjeldahl-Gunning-Arnold method for nitrogen. Sugars were run by the Scales' method which has tentative A.O.A.C. approval (A.O.A.C., 1940). Oil was determined by an extraction with petroleum ether in a Butt apparatus (American Oil Chemists' Society method for cottonseed). Starch was determined by the polarimetric method using either the tentative A.O.A.C. method (Lapp, 1944) or

TABLE I

AVERAGE CORNCOB YIELDS OF FARM-GROWN DOUBLE-CROSS HYBRIDS, BY VARIETY AND SOURCE, 1941 CROP

Hybrid variety	Source									Summary		
	Illinois			Iowa			Indiana					
	No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn		No. samples	Cob yields per bushel of shelled corn	
		lb	%		lb	%		lb	%		lb	%
U. S. 13	8	10.0	15.1	3	10.3	15.5	6	10.0	15.1	17	10.0	15.2
U. S. 35	5	9.3	14.2	2	10.0	15.2	3	9.7	14.8	10	9.6	14.6
U. S. 44	3	10.1	15.3	5	9.9	15.0	—	—	—	8	10.0	15.1
Ill. 960	2	7.8	12.2	6	9.6	14.6	—	—	—	8	9.1	14.0
Ill. 200	3	10.1	15.3	—	—	—	3	10.9	16.3	6	10.5	15.8
Ill. 21	5	9.3	14.3	—	—	—	—	—	—	5	9.3	14.3
Ill. 784	5	9.4	14.4	—	—	—	—	—	—	5	9.4	14.4
Ill. 877	4	8.7	13.4	—	—	—	—	—	—	4	8.7	13.4
Iowa 13	—	—	—	6	9.3	14.3	—	—	—	6	9.3	14.3
Iowa 939	—	—	—	6	10.6	15.9	—	—	—	6	10.6	15.9
U. S. 63	—	—	—	3	10.1	15.3	—	—	—	3	10.1	15.3
Ind. 608	—	—	—	—	—	—	7	10.2	15.4	7	10.2	15.4
Ind. 610	—	—	—	—	—	—	3	9.7	14.7	3	9.7	14.7
Ind. 613	—	—	—	—	—	—	3	9.3	14.2	3	9.3	14.2
Ind. 844	—	—	—	—	—	—	9	9.6	14.6	9	9.6	14.6
Ind. 416	—	—	—	—	—	—	2	9.3	14.3	2	9.3	14.3
Weighted average	35	9.5	14.4	31	9.9	15.1	36	9.9	15.0	102	9.7	14.8

the Clendenning modification (Earle and Milner, 1944). The specific rotation of starch was considered to be 203°.

While the grain samples from farm fields were not produced under uniform soil and climatic conditions, it is believed that the data presented will give some indication of the composition of the corn produced under ordinary farm conditions. In the case of some of the hybrid varieties it was not practicable to collect a sufficient number of samples for their proper evaluation.

Proportion of Cobs to Shelled Corn

Data on the proportionate yield of cobs to shelled corn for the ear corn samples are presented in Table I. These data, obtained by shelling 15-pound samples, show variety averages by states. All ears were well filled and practically free of husks, a condition which seldom occurs commercially. Shelling was finished by hand in order to make a perfect separation of grain and cobs. The over-all average shown is 9.7 pounds of cobs per bushel (56 pounds) of shelled corn. The extremes in cob yields of individual samples were 7.8 pounds and 10.9 pounds. The samples from Illinois averaged slightly lower in yield of cobs than those from the other states. Comparison of varieties for all states shows Illinois Hybrid 877 lowest with an average yield of 8.7 pounds and Illinois Hybrid 960, second lowest with an average yield of 9.1 pounds. The varieties with the highest average cob yields were Iowa Hybrid 939 and Illinois Hybrid 200 with yields of 10.6 and 10.5 pounds, respectively.

Chemical Results and Discussion

Analytical data showing variety averages and averages of all shelled corn samples by states are presented in Table II. Results on samples from Ohio and Oklahoma are included in the table, but the number of samples from these states is too small to be representative and they are not considered in the following comparison by states. The Illinois samples averaged lowest in weight per 1,000 kernels and highest in weight per bushel and in oil and sugar contents. The Indiana samples averaged highest in weight per 1,000 kernels and in ash and protein contents, and lowest in starch. The Iowa samples averaged highest in starch content and lowest in ash.

It should be emphasized that some of the differences mentioned in the preceding paragraph and also many of those in the following paragraph are so small that they can be considered only as possible trends. The data presented by Doty *et al.* (1943) indicate that seasonal differences may be even greater than some of the differences mentioned here.

Average analyses of all samples collected for each variety, together with averages of all samples and relative rank of each variety, are presented in Table III. Illinois Hybrid 200 averaged highest in both protein and oil and lowest in starch content. Illinois Hybrid 21 averaged highest in sugar, Illinois Hybrid 960 highest in starch, U. S. Hybrid 44 highest in weight per bushel, and Indiana Hybrid 425 highest in ash content. Iowa Hybrid 13 averaged highest in weight per 1,000 kernels and lowest in protein content. Iowa Hybrid 939 averaged lowest in weight per bushel and in oil content. Illinois Hybrid 877 averaged lowest in weight per 1,000 kernels, Illinois Hybrid 784 lowest in ash content, and U. S. Hybrid 63 lowest in sugar content.

In connection with the data in Table III, it is significant that in every instance the varieties with high protein content had low starch content and vice versa.

The oil content is mainly a varietal characteristic, although some

TABLE II

PHYSICAL AND CHEMICAL COMPOSITION OF FARM-GROWN DOUBLE-CROSS HYBRID CORN VARIETIES, BY VARIETY AND STATE, 1941 CROP

Hybrid variety	No. of samples	Air-dry basis		Moisture-free basis				
		Wt. per bushel	Wt. per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		lb	*g*	%	%	%	%	%
Samples from Illinois:								
U. S. 13	11	60.7	319.3	1.44	10.7	4.7	1.99	69.6
U. S. 35	7	59.3	296.4	1.43	10.7	4.7	1.96	69.6
U. S. 44	4	61.2	308.8	1.43	10.5	4.9	2.17	70.3
U. S. 63	1	59.5	302.0	1.39	10.4	4.7	1.79	70.9
Illinois 21	10	60.1	329.5	1.45	11.4	4.8	2.14	68.9
Illinois 200	8	60.3	308.0	1.46	11.1	5.6	2.14	68.6
Illinois 784	10	60.3	310.6	1.34	10.3	4.9	2.00	69.4
Illinois 877	9	59.9	271.7	1.38	10.6	5.2	1.93	69.7
Illinois 960	5	59.7	298.3	1.43	10.0	4.6	1.93	71.4
Iowa 939	1	57.0	278.4	1.38	9.5	4.0	1.84	71.6
Indiana 416	1	58.3	290.2	1.40	10.2	4.6	1.58	71.9
Indiana 608	1	58.4	304.8	1.48	10.1	4.0	2.03	71.2
Weighted average	68	60.1	305.9	1.42	10.7	4.9	2.02	69.6
Samples from Iowa:								
U. S. 13	3	60.7	305.6	1.29	10.2	4.6	1.85	71.4
U. S. 35	2	59.2	288.6	1.45	9.7	4.4	1.98	71.1
U. S. 44	5	60.5	329.8	1.46	10.5	5.0	2.09	70.4
U. S. 63	5	59.1	281.8	1.36	9.8	4.5	1.87	72.1
Illinois 960	6	60.0	264.7	1.32	10.1	4.6	1.91	71.5
Iowa 13	7	59.5	338.8	1.46	9.8	4.2	2.13	71.8
Iowa 939	8	58.2	343.6	1.36	11.3	3.8	1.94	70.3
Indiana 608	1	58.3	286.5	1.28	10.4	4.0	2.05	71.3
Weighted average	37	59.4	312.1	1.38	10.3	4.4	1.98	71.2

TABLE II—*Continued*

Hybrid variety	No. of samples	Air-dry basis		Moisture-free basis				
		Wt. per bushel	Wt. per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		lb	*g*	%	%	%	%	%
Samples from Indiana:								
U. S. 13	5	59.8	324.8	1.43	10.5	4.6	1.93	70.1
U. S. 35	3	59.7	302.3	1.45	10.9	4.8	2.02	69.4
Illinois 200	3	60.1	334.0	1.49	12.6	5.3	2.06	67.6
Iowa 939	1	58.2	315.0	1.37	11.2	4.0	2.03	70.7
Indiana 416	6	60.9	285.1	1.55	11.3	4.8	1.93	69.2
Indiana 425	1	58.9	323.1	1.59	11.1	4.9	1.91	69.2
Indiana 610	5	58.7	333.0	1.44	11.5	4.8	2.06	69.0
Indiana 613	4	58.6	300.0	1.43	10.8	4.1	1.98	70.6
Indiana 608	9	58.8	304.4	1.44	11.2	4.4	2.00	69.8
Indiana 844	12	59.5	329.8	1.41	11.2	4.6	1.96	69.5
Weighted average	49	59.4	315.2	1.45	11.2	4.6	1.98	69.5
Samples from Ohio:								
Indiana 416	1	58.2	312.5	1.29	10.9	4.8	1.81	69.7
Indiana 425	1	59.5	294.1	1.48	11.1	4.7	1.84	70.1
Indiana 610	1	57.0	313.0	1.44	10.8	4.9	1.93	69.7
Weighted average	3	58.2	306.5	1.37	10.9	4.8	1.86	69.8
Samples from Oklahoma:								
U. S. 35	2	54.4	265.8	1.58	11.3	4.4	1.98	68.9
All samples (159), all varieties, all locations:								
Average		59.6	309.7	1.42	10.8	4.7	1.99	70.0
Maximum		62.5	392.2	1.67	14.1	5.7	2.50	73.4
Minimum		53.1	224.3	1.20	8.3	3.6	1.58	65.8

variation may be attributed to other causes. Only one sample of Illinois Hybrid 200 out of 11 analyzed was below 5% in oil content, while the highest out of 10 samples of Iowa Hybrid 939 had only 4.1% oil. Of the 19 samples of U. S. Hybrid 13 analyzed, 15 were within 0.2% of its average. Illinois Hybrid 200 was outstandingly higher in oil content, and Iowa Hybrid 939 outstandingly lower than the other varieties.

Starch, sugar, ash, and protein may be varietal characteristics, but more data will need to be accumulated over a number of years before definite conclusions can be drawn.

Analytical Data from Hybrid Varieties Produced at Experiment Stations

Samples of five of the double-cross hybrid varieties collected from farm fields also were obtained from the Illinois, Iowa, and Indiana

TABLE III

PHYSICAL AND CHEMICAL COMPOSITION OF FARM-GROWN DOUBLE-CROSS HYBRID CORN VARIETIES, VARIETY AVERAGES FOR ALL LOCATIONS, 1941 CROP

Hybrid variety	No. of samples	Air-dry basis				Moisture-free basis									
		Wt. per bushel		Wt. per 1,000 kernels		Ash		Protein (N × 6.25)		Oil		Sugar		Starch	
		lb	*rank*	*g*	*rank*	%	*rank*	%	*rank*	%	*rank*	%	*rank*	%	*rank*
U. S. 13	19	60.5	2	317.3	7	1.42	11	10.5	12	4.7	9	1.95	11	70.1	7
U. S. 35	14	58.7	13	292.9	13	1.46	4	10.7	10	4.6	10	1.98	8	69.8	9
U. S. 44	9	60.8	1	320.4	6	1.44	7	10.5	12	5.0	3	2.13	2	70.3	6
U. S. 63	6	59.2	11	285.2	15	1.37	14	9.9	16	4.5	13	1.86	17	71.9	2
Illinois 21	10	60.1	6	329.5	5	1.45	6	11.4	2	4.8	5	2.14	1	68.9	16
Illinois 200	11	60.3	3	315.1	8	1.47	3	11.5	1	5.5	1	2.12	4	68.3	17
Illinois 784	10	60.3	3	310.6	9	1.34	17	10.3	14	4.9	4	2.00	7	69.4	14
Illinois 877	9	59.9	7	271.7	17	1.38	13	10.6	11	5.2	2	1.93	13	69.7	10
Illinois 960	11	59.9	7	280.0	16	1.37	14	10.0	15	4.6	10	1.92	14	72.0	1
Iowa 13	7	59.5	9	338.8	1	1.46	4	9.8	17	4.2	15	2.13	2	71.8	3
Iowa 939	10	58.1	17	334.2	2	1.37	14	11.1	5	3.9	17	1.94	12	70.5	5
Indiana 416	8	60.2	5	289.2	14	1.50	2	11.1	5	4.8	5	1.88	15	69.6	12
Indiana 425	2	59.2	11	308.6	10	1.54	1	11.1	5	4.8	5	1.88	15	69.7	10
Indiana 608	11	58.7	13	304.5	11	1.43	9	11.0	8	4.4	14	2.01	6	70.0	8
Indiana 610	6	58.4	16	329.7	4	1.44	7	11.4	2	4.8	5	2.04	5	69.1	15
Indiana 613	4	58.6	15	300.0	12	1.43	9	10.8	9	4.1	16	1.98	8	70.6	4
Indiana 844	12	59.5	9	329.8	3	1.41	12	11.2	4	4.6	10	1.96	10	69.5	13
Weighted average (159)		59.6		309.7		1.42		10.8		4.7		1.99		70.0	

Agricultural Experiment stations. The data for these samples are presented in Table IV, together with data of samples of the same varieties collected from farm fields in the states specified. The experiment station samples, except for Iowa Hybrid 939 grown in Illinois, were consistently lower in ash and protein content, and higher in starch content than were the farm-produced samples, but in other factors there were no consistent differences.

Summary

The ash, protein, oil, sugar, and starch contents were determined for 17 hybrid corn varieties (159 samples) grown in farm fields in 1941, and for five of these varieties grown at the Iowa, Illinois, and Indiana Agricultural Experiment stations.

Variations were found among samples for all chemical constituents for which analyses were made. These differences can be attributed to heredity and/or environment. Oil content was largely a varietal characteristic. In every instance, samples having a high protein content were low in starch content and vice versa. Ash and protein contents were lower and starch higher in experiment station samples than in those grown on farms.

TABLE IV

PHYSICAL AND CHEMICAL COMPOSITION OF DOUBLE-CROSS HYBRID CORN VARIETIES—FARM AND EXPERIMENT STATION SAMPLES, 1941 CROP

Hybrid variety and source	Kind of production	No. samples analyzed	Air-dry basis		Moisture-free basis				
			Wt. per bushel	Wt. per 1,000 kernels	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
			lb	*g*	%	%	%	%	%
U. S. 13									
Illinois	Farm field	11	60.7	319.3	1.44	10.7	4.7	1.99	69.6
Illinois	Experiment station	3	57.5	308.1	1.26	10.6	4.7	2.01	71.6
Iowa	Farm field	3	60.7	305.6	1.29	10.2	4.6	1.85	71.4
Iowa	Experiment station	3	60.6	314.3	1.28	9.5	4.7	2.02	71.7
Indiana	Farm field	5	59.9	320.0	1.45	10.4	4.7	1.93	70.3
Indiana	Experiment station	3	60.5	295.1	1.26	9.4	4.7	2.08	71.8
U. S. 44									
Illinois	Farm field	4	61.2	308.8	1.43	10.5	4.9	2.17	70.3
Illinois	Experiment station	3	60.0	305.7	1.31	9.8	5.2	2.02	72.2
Iowa	Farm field	5	60.5	329.8	1.46	10.5	5.0	2.09	70.4
Iowa	Experiment station	3	60.7	303.7	1.47	10.0	4.9	2.21	74.2
Illinois 784									
Illinois	Farm field	10	60.3	310.6	1.34	10.3	4.9	2.00	69.4
Illinois	Experiment station	3	57.8	306.0	1.29	9.8	5.3	3.19	70.5
Iowa 939									
Illinois	Farm field	1	57.0	278.4	1.38	9.5	4.0	1.84	71.6
Illinois	Experiment station	3	59.0	324.8	1.36	11.2	5.3	2.09	70.6
Iowa	Farm field	8	58.2	343.6	1.36	11.3	3.8	1.94	70.3
Iowa	Experiment station	3	58.7	306.2	1.30	9.8	3.9	1.85	72.3
Indiana	Farm field	1	58.2	315.0	1.37	11.2	4.0	2.03	70.7
Indiana	Experiment station	3	58.6	315.7	1.32	9.6	4.0	2.08	71.6
Indiana 884									
Indiana	Farm field	12	59.5	329.8	1.41	11.2	4.6	1.96	69.5
Indiana	Experiment station	3	58.6	315.7	1.32	9.6	4.0	2.08	71.6

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LOAF VOLUME POTENTIALITIES, BUFFERING CAPACITY, AND OTHER BAKING PROPERTIES OF SOY FLOUR IN BLENDS WITH SPRING WHEAT FLOUR [1]

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It has long been recognized that soybean proteins effectively supplement wheat proteins in animal nutrition and greatly increase their nutritive value by the addition of lysine and other amino acids in which wheat proteins are low. Recently much attention has been given to the possibility of improving the nutritive value of bread, macaroni, and other cereal products by supplementing with small amounts of soybean flour. These efforts have met with limited success, the major objections to the use of soy flour in bread being the undesirable bean flavor, the color imparted to the crumb, and the low loaf volume. Considerable work has been done by Beckel and Smith (1944) in overcoming the first two objections.

It seemed probable that loaf volumes might be improved if more was known concerning the baking properties of soybean-wheat flour mixtures. Accordingly, studies with such mixtures or blends have been carried out in the Federal Soft Wheat Laboratory at Wooster, Ohio. This paper describes certain baking properties of these blends and shows how loaf volume and crumb grain are affected when certain alterations in the baking formula and techniques are made on the basis of these properties.

Materials and Methods

Two hexane-extracted soy flours (No. 1 and No. 2) and one extracted with alcohol (A), provided by the Northern Regional Research

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Oil and Protein Division, Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture; and the Department of Agronomy, Ohio Agricultural Experiment Station.

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Laboratory, were blended in varying percentages with a commercially milled and bleached hard red spring wheat flour containing 15.0% protein. The blends contained 2, 4, 6, and 8% of soy flour. Each of these and wheat flour alone were baked with 0, 1, 2, 3, and up to 8 mg of potassium bromate per 100 g of flour. Additional ingredients used in all bakes were 6 g sugar, 1½ g salt, 3 g shortening, 2 g yeast, ¼ g malt syrup (120°L), and water as needed. Milk solids were included in the studies with two of the soy flours. An optimum mixing time with the straight-dough procedure and a 3-hour fermentation at 30°C were employed. All punching and panning were performed mechanically. Baking time was 25 minutes at 221°C. Bakings were replicated at least twice. A third replicate was made when loaf volumes differed by more than 25 cc.

Experimental Results

The absorption, mixing time, and the potassium bromate requirement for the wheat flour and the various blends with soy flour are shown in Table I and the loaf volumes, crumb grain scores, and crumb color scores are shown graphically in relation to the potassium bromate requirements in Figures 1 to 3. The bromate requirement given in Table I is the amount of potassium bromate required to produce approximately the maximum loaf volume as shown in Figure 1.

The wheat flour without soy flour and without milk solids produced the largest loaves (Figure 1A) with about 1 mg of potassium bromate. When this flour was blended with various quantities of soy flour, however (Figure 1B to 1M), and baked into bread by the same formula (1 mg of bromate and no milk solids) the loaf volumes were in most instances reduced. For example, 6% and 8% of soy flour No. 1 reduced the loaf volume about 25 cc and 45 cc, respectively, and the same concentrations of alcohol-extracted soy flour (A) depressed loaf volumes 65 cc and 85 cc, respectively. The reductions from the use of soy flour No. 2 were even greater.

The decrease, in general, varied with the amount of soy flour used. With additional increments of potassium bromate, however, the loaf volumes increased to an optimum that was either approximately equal to or considerably greater than that for wheat flour alone, depending on the quantity and kind of soy flour used. Thus, with no milk solids in the formula, the addition of 2% of soy flour No. 1 (Figure 1B) resulted in a loaf volume increase of 70 cc when the quantity of bromate was increased from 1 to 3 mg. Additional amounts of soy flour No. 1 up to the maximum of 8% used in this study also produced loaves larger than those obtained from wheat flour alone, provided additional quantities of potassium bromate were

TABLE I

ABSORPTION, MIXING TIME, AND POTASSIUM BROMATE REQUIREMENT FOR HARD RED SPRING WHEAT FLOUR DOUGHS CONTAINING VARYING AMOUNTS OF SOY FLOURS, WITH AND WITHOUT NONFAT MILK SOLIDS

Kind and percent of soy flour in blends	Baking absorption		Mixing time		Potassium bromate requirement	
	No milk solids	4% nonfat milk solids	No milk solids	4% nonfat milk solids	No milk solids	4% nonfat milk solids
	%	%	*min.*	*min.*	*mg*	*mg*
No. 1						
0	68.5	70.5	3½	3½	1	1
2	70.5	72.5	3½	3½	2	3
4	72.5	74.5	3⅝	3⅝	3	5
6	74.5	76.5	3⅝	3⅝	4	5
8	76.5	78.5	3⅞	3⅞	5	4
A						
2	72.0	74.0	3½	3½	1	3
4	75.5	77.5	3⅝	3⅝	2	3
6	79.0	81.0	3⅝	3⅝	3	3
8	82.5	84.5	3⅞	3⅞	3	3
No. 2						
2	71.5	—	3½	—	2	—
4	74.5	—	3½	—	2	—
6	77.5	—	3⅝	—	3	—
8	80.5	—	3½	—	4	—

used. The amount of bromate required for approximately optimum loaf volume increased, in general, with the concentration of soy flour. Thus, 5 mg were required for 8% of soy flour No. 1 in contrast to only 1 mg for wheat flour alone. The requirements for the blends of all three soy flours are given in Table I.

Another property of soy flour equally as obvious and striking as "bromate requirement" and "loaf volume imparting potentialities" is the capacity to buffer the oxidative effects of overbromating. All three of these properties are associated with nonfat milk solids, as illustrated by the data in Figure 1A, and are important in test baking and most commercial shop practices. A comparison of these two materials, therefore, is pertinent to a proper evaluation of soy flour. The buffering effect of the latter is well illustrated by the no-milk blend containing 4% of No. 1 soy flour. The maximum loaf volume was secured with 4 mg of potassium bromate (dots, Figure 1C). It will be noted that 5 and 6 mg reduced the loaf volume even less than did an equal excess of potassium bromate when using 4% of nonfat milk solids (circles, Figure 1A).

The same data show that 4% soy flour No. 1 produced greater loaf volume increases than did an equal quantity of nonfat milk solids,

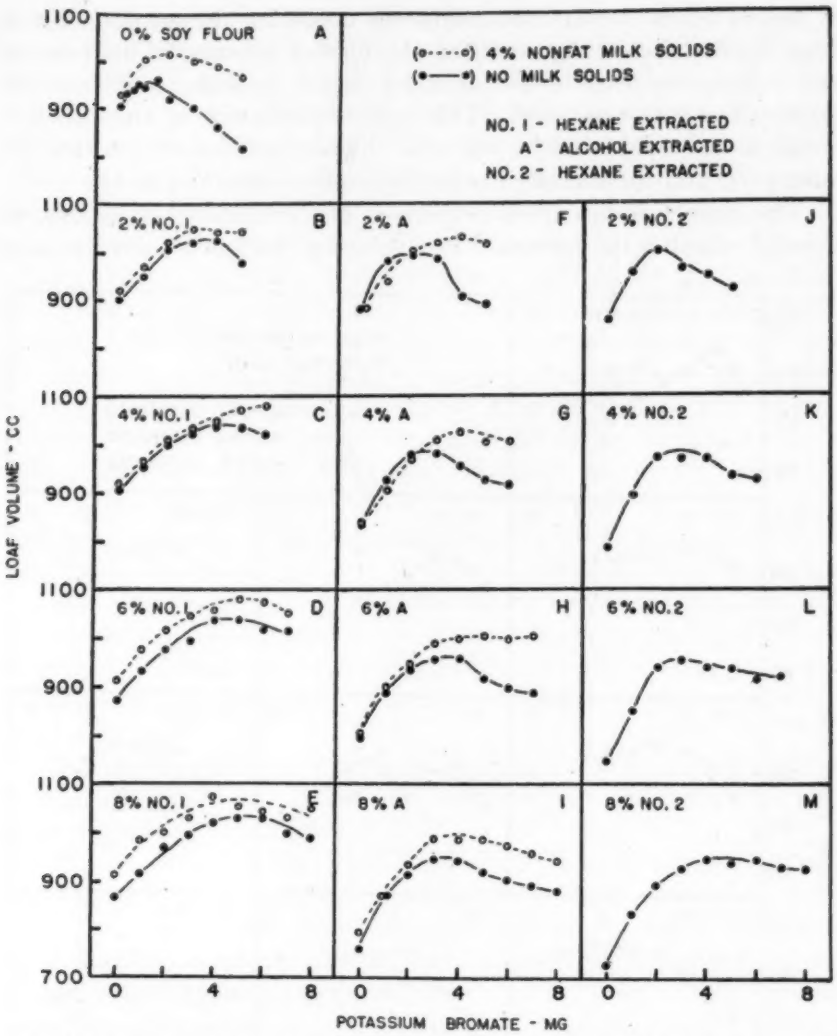

Fig. 1. Loaf volumes for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and potassium bromate, and with and without nonfat milk solids.

provided additional potassium bromate was used. For example, 4% of soybean flour No. 1 increased the loaf volume 80 cc compared to that for the spring wheat flour alone; whereas the same amount of nonfat milk solids (4%) increased loaf volume only about 55 cc, comparisons in both cases being with formulas containing the optimum amounts of potassium bromate. Both the alcohol-extracted and commercial soy flour No. 2 had somewhat the same properties as did soy flour No. 1, but their effects in the blends were less, both in regard to increasing the loaf volume and to buffering effects.

When 4% of nonfat milk solids was used with the soy flour–wheat flour blends (circles, Figure 1B to 1I), further substantial increases in loaf volume resulted in all instances, again provided sufficient potassium bromate was used. This additive behavior of the two materials is also evidenced by the more highly buffered curves, for the most part, and the increased bromate requirements (Table I).

The crumb grain curves (Figure 2) are principally of interest in showing whether the increased loaf volumes of Figure 1 were secured

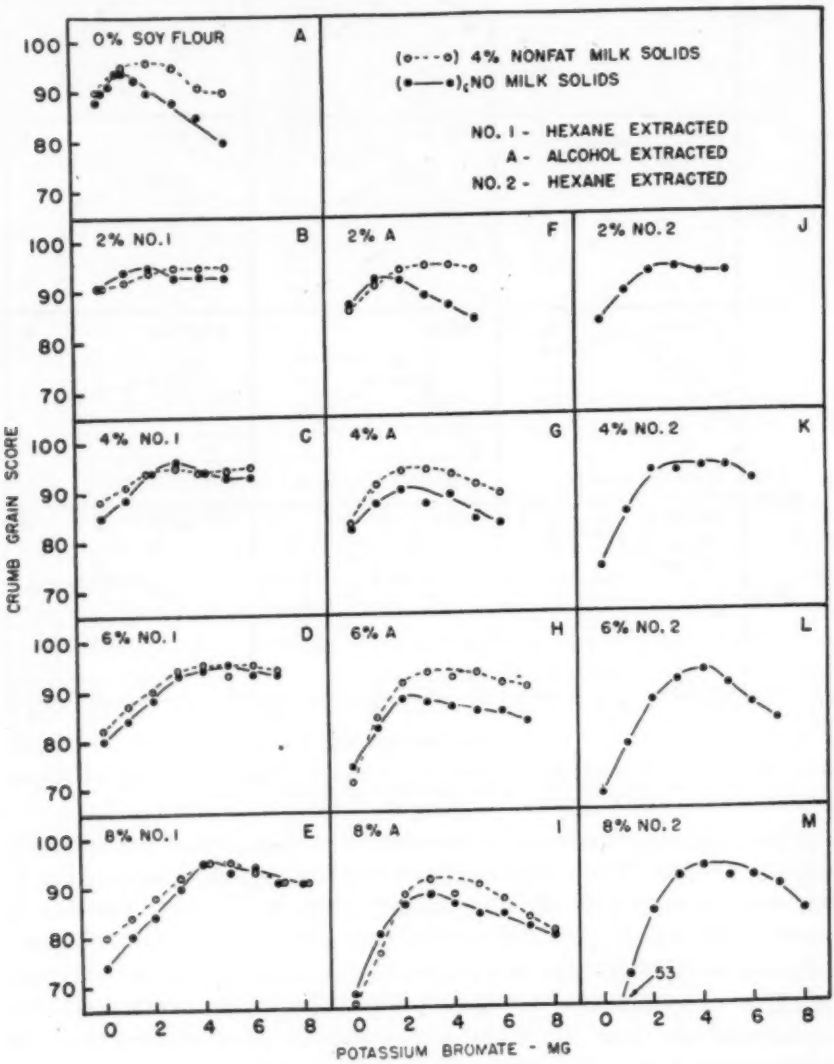

Fig. 2. Crumb grain scores for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and potassium bromate, and with and without nonfat milk solids.

at the expense of the crumb grains of the finished loaf. It will be noted that adding each of the soybean flours decreased the crumb grain scores as compared with the wheat flour alone when only 1 mg of potassium bromate was used, and this depressing effect, also noted for loaf volume, increased with the concentration of soybean flour. These scores, however, gradually improved as the quantity of bromate increased, in the same manner as was noted for loaf volume, so that the loaf volume curves and crumb grain curves roughly parallel each other. Most important, the optimum crumb grains and optimum loaf volumes were produced with the same quantity of bromate in the formula. In addition, the optimum crumb grains for the blends with soy flour No. 1 were fully as good as those obtained with wheat flour alone. In brief, the effects on crumb grain scores are so similar to those on loaf volume that the latter may for all practical purposes be used alone to evaluate these effects. It should be pointed out, nevertheless, that with no more than 4% of the alcohol-extracted soy flour and no nonfat milk solids, most crumb grains are either of questionable quality or are unsatisfactory. With milk solids, however, the crumb grains for all percentages of alcohol-extracted soy flour were considerably improved and satisfactory with the possible exception of the 8% blend. In commercial bread production, therefore, it appears that this alcohol-extracted type of soy flour should be used in conjunction with milk solids.

The crumb color scores corresponding to the optimum loaf volumes and crumb grains for the various blends of soy and wheat flour are shown graphically in Figure 3.

The crumb colors for 2% of soy flour No. 1 without milk solids are detectably whiter than for the wheat flour alone, probably because of the higher volumes which are accompanied by a somewhat finer crumb grain structure. With concentrations greater than 2% the crumb color became progressively more creamy-gray to dull-brownish with increasing concentration of soy flour, each 2% corresponding to an average decrease of 8 points in color score. The effect of the alcohol-extracted flour is about the same and that of the commercial soy flour No. 2 is somewhat greater.

With the scoring used in this study a crumb color value of about 100, which characterizes the control, is considered excellent. It is questionable whether a crumb color of 90 would be noticed by the average consumer of white bread and scores as low as 75 to 80 would go unnoticed by many. Even if observed, many would not object so long as the bread was properly developed and had a suitable texture and crumb grain.

The water absorption and mixing times for the hard red spring

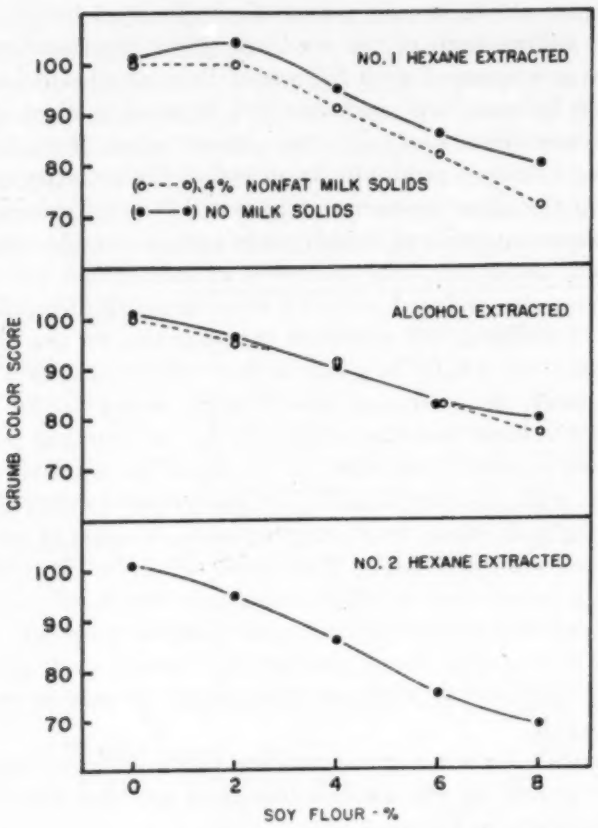

Fig. 3. Crumb color scores for a hard spring wheat flour (15% protein) baked with varying increments of soy flours and with and without nonfat milk solids.

flour alone and when blended with the various percentages of the different soy flours both with and without milk solids are given in Table I. It was found necessary to add 1.0, 1.75, and 1.5% additional water for each percent of soy flour No. 1, A, and No. 2, respectively.

The first 2% of soy flour added did not alter mixing time appreciably. For each succeeding 2% of soy flour added, an increase of $\frac{1}{4}$ minute was required except for soy flour No. 2 which required an increase of only $\frac{1}{8}$ minute.

General Discussion

The results obtained in this study indicate not only that excellent bread may be made from wheat-soy flour blends including up to 8% of the latter but that loaf volumes and texture-qualities on which consumer preference seems largely to depend may actually be improved. To secure this desirable result, however, it is necessary to

use larger quantities of potassium bromate in the baking formula than is customary. In general, the amount of bromate required increases with the percentage of soy flour in the blend. In these studies the bromate requirement for blends including 8% soy flour varied from three to five times that needed for the wheat flour alone.

These results also indicate that soy flour may be used in place of nonfat milk solids and have about the same buffering effect on potassium bromate.

The superior baking properties and potentialities of the hexane-extracted soy flour No. 1 suggest that the solvent and/or extraction technique are of extreme importance in the processing of soybeans into flour for blending with wheat flours, assuming, of course, that the soybeans used in the processing of all three flours were of comparable quality.

The work reported herein should be repeated with other types of flour, particularly hard red winter. If a hard winter flour of comparable protein quality and quantity was used, there seems to be no reason to anticipate results substantially different from those shown with the hard spring wheat flour used in this study, except that the quantity of bromate for optimum results probably would be increased by 1 or 2 mg because of the greater average bromate requirement of the hard winter wheats.

Another potentially important result of this study may be in suggesting a sound procedure for testing the effect of soy flour on bread quality and especially the relative value of different soy flours to be used for this purpose. There is reason to believe that unfavorable results in the past with wheat-soy flour blends may have been due to inadequate baking formulas, especially in regard to the amount of potassium bromate. It seems also that the effect of soy flour on absorption and mixing time must be considered if dependable results are to be secured.

Summary

A commercially milled and bleached hard red spring wheat flour containing 15% protein was baked with various increments up to 8% of each of three different soy flours. Various increments of potassium bromate up to 8 mg per 100 g of flour were used. Absorption and mixing time were varied to suit the needs of each blend. Bakings for two of the soy flour blends were made with and without 4% milk solids.

Excellent bread can be made with blends containing up to 8% soy flour with hard red spring wheat flour, the quality of the bread as measured by loaf volume and crumb grain being even better than for

wheat flour alone provided the quantity of potassium bromate used in the baking formula is increased according to the amount of soy flour used. Absorption and mixing time increased with the amount of soy flour used.

A hexane-extracted flour buffered the effects of excess potassium bromate fully as effectively as nonfat milk solids. The similarity of soy flour to milk solids is of significance from the standpoint of baking technology.

Crumb color of the bread, in general, became progressively more creamy-gray with increasing amounts of soy flour. The color change produced by adding as much as 4% of soy flour probably would not be noticed by most consumers, and would not be objectionable to many others.

Of the three soy flours used in this study a hexane-extracted flour proved the best. In making the comparison, however, the taste and flavor factors were not considered. Soy flours have specific baking properties and potentialities in blends with wheat flour. These potentialities remain hidden, however, unless suitable alterations are made in the baking formula and technique.

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EFFECT OF YEAST, BROMATE, AND FERMENTATION ON BREAD CONTAINING SOY FLOUR [1]

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The proteins of the soybean are high in lysine, and the addition of soy flour to white flour improves the quality of the flour proteins (Carlson *et al.*, 1946; Harris *et al.*, 1944; Jones and Divine, 1944; Volz *et al.*, 1945). But the addition of soy flour to bread without other change in formula or handling is often accompanied by a deterioration in the quality of the bread volume, external appearance, grain, and texture and color of crumb.

The addition of any nonglutinous substance to a dough acts as a diluent of the gluten. This effect, using starch, was studied by Johnson and Bailey (1925). Bailey, Capen, and LeClerc (1935) noted an

[1] Contribution No. 120, Department of Milling Industry, Kansas Agricultural Experiment Station.

acceleration of fermentation when soy flour was added to a dough. Bohn and Favor (1945) reported the effects of soy flour on the absorption, mixing tolerance, dough fermentation, gas production, and staling of bread. They found that full-fat soy flour had a water absorption of about 85% and extracted soy flour of 110% when used in doughs. Low-fat soy flour and, to a lesser degree, full-fat soy flour reduced the mixing time, as well as the dough stability upon overmixing. With 3% additions of soy flour, the effect on gas production was negligible. Soy flour acted on a fermenting dough similarly to glutathione. Dough properties were improved by the use of sufficient oxidizing agents, such as bromate and iodate. Soy flour retarded the staling of bread.

Some of the factors which may be varied in breadmaking are the fermentation time and the percentage of yeast and potassium bromate. The object of this work was to observe the effects of variations of these factors on the baking properties of wheat- and soy-flour mixtures.

Materials and Methods

Full-fat and low-fat (both extracted and expeller) soy flours were obtained from the five largest producers. The analyses of these were very uniform in each class.

These samples were given preliminary baking tests by the standard A.A.C.C. procedure. It was found that full-fat and extracted soy flours had very similar baking characteristics. Expeller soy flours showed inferior baking characteristics. Composition of the representative low-fat (extracted) soy flour selected for study was: moisture, 7.0%; ether extract, 1.0%; protein (N × 6.25), 51.0%; ash, 5.5%.

The wheat flour was a standard patent Kansas flour of 0.48% ash and 13.0% protein from the 1943 crop. All doughs were made up using 6% sugar, 1.5% salt, and 0.25% 120°L malt. The absorption was that found to be optimum by the mixograph. Immediately after mixing, 170 g of dough was scaled. Punching and molding were performed by machine to obtain uniformity. Low form pans were used. The volume and weight of the loaves were taken immediately after removal from the oven. Texture, grain, and crumb color were scored the following day.

Potassium bromate was varied in amounts of 0, 1, 3, 5, 7, 9, and 11 mg per 100 g flour and 1%, 2.5%, and 4.0% yeast were used. The percentages of soy flour were 0, 1.5, 3.0, 4.5, 6.0, and 7.5. The fermentation times (to molding) were 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours.

Three variations of yeast, six of soy flour, six of bromate, and seven of fermentation make a total of 756 possible variations. Since

the work was performed in duplicate, a total of 1,512 loaves was produced.

Straight doughs were used in this work. While it is realized that most commercial bread is made by the sponge method, it is fairly generally agreed that indications given by the straight dough method on the effects of variations in bromate, fermentation time, etc., apply to sponge doughs, when properly interpreted (Shellenberger and Ziemke, 1939).

To determine the effect of dilution of the gluten by soy flour on bread volume, the standard A.A.C.C. method was used. In control loaves 3, 6, and 9% of starch were used and compared to the same quantities of low-fat (extracted) and full-fat soy flours.

The bread was scored for the customary factors. The volume of loaves was reported in cubic centimeters. Crumb texture was scored on the basis of silkiness and compressibility. A standard (optimum) texture was scored 95. Textures under 80 were poor, being harsh and open, with round, thick cell walls. While the word "open" usually refers to grain rather than texture, all texture scores under 80 indicated a poor grain. Grain was scored in connection with texture. A standard grain was scored 2.00, a close grain 3.00, and an open grain 1.00. Grain scores were not too significant, but are shown in Table IV, which gives averages of all the data obtained. Fermentation and baking loss were obtained by weighing 170 g of dough out of the mixer, and weighing the bread as it came from the oven.

Discussion of Results

Figure 1 shows the effect of gluten dilution on volume. Soy flour reduces the volume of bread more than can be accounted for by

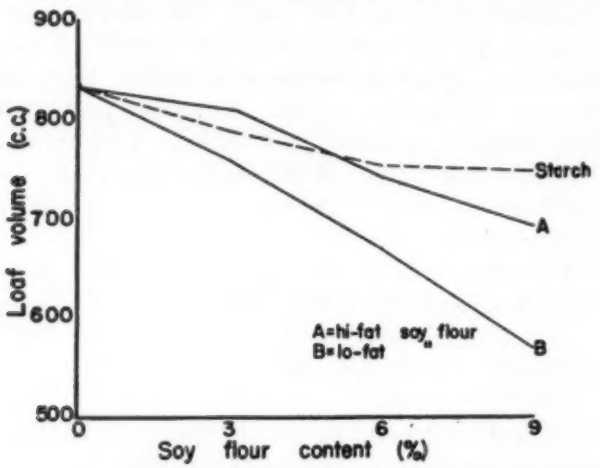

Fig. 1. Effect of soy flour on loaf volume.

straight dilution. This would indicate that soy flours contain some factor which has a deteriorating effect on bread volume.

Figure 2 shows visually the effect of some of the variables studied on loaves containing 9.0% soy flour. This is a higher level than was used in this study, and higher than would be used in commercial white bread. Even with this high percentage of soy flour, bread made under optimum conditions closely resembles the check (nonsoy) loaves.

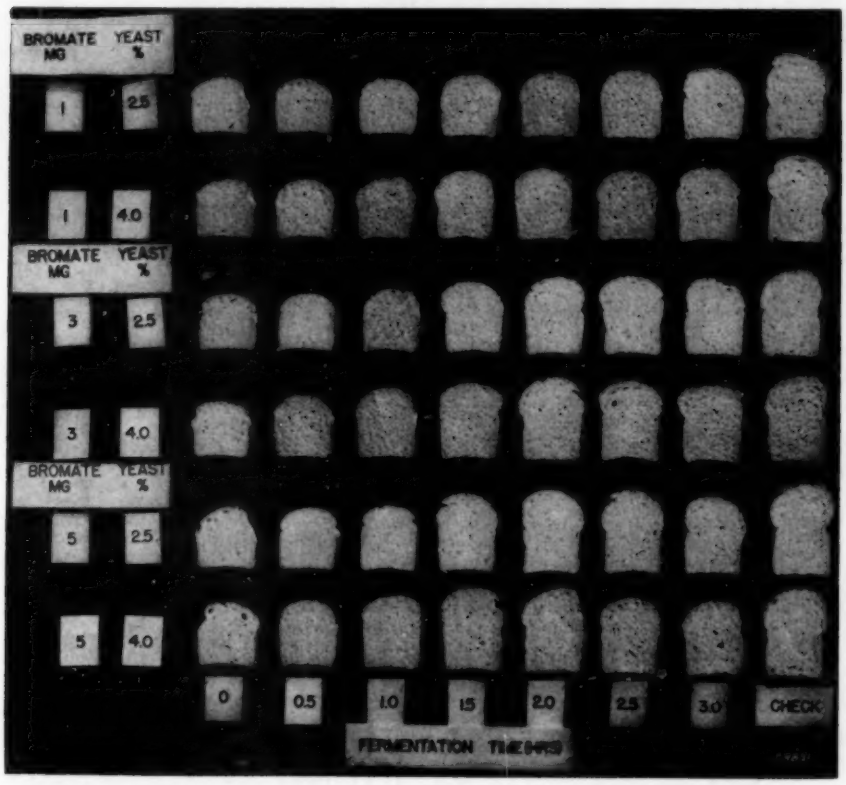

Fig. 2. Internal characteristics of typical loaves from formula procedure study—9.0% soy flour used in all but the check loaves.

It would be too cumbersome to present the data on all the loaves produced. For brevity in Tables I, II, and III, the extremes of bromate used have been omitted, along with the data for some of the intermediate fermentation times and soy flour percentages.

The data in these tables cover the range of the optimum conditions for soy flour within the limits of practical, commercial formulas.

Effect of Soy Flour on Loaf Volume. For optimum volume, bread containing 1.5% of soy flour requires about 2 mg more bromate per

TABLE I

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 1% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	555	492	535	455	472	512	428	455	465
	Texture	50.0	48.5	68.5	54.0	61.0	50.0	45.0	46.5	40.0
	Ferm. and bake loss %	10.9	10.7	12.2	11.4	10.7	11.1	10.7	10.6	10.7
2 hr.	Volume cc	572	598	620	532	565	592	492	570	522
	Texture	82.5	76.5	75.5	82.0	78.5	81.5	67.5	73.0	49.0
	Ferm. and bake loss %	12.2	12.8	12.1	12.8	11.8	12.7	10.9	11.9	11.7
3 hr.	Volume cc	666	693	665	648	640	660	602	613	580
	Texture	86.0	87.0	72.5	85.0	79.0	70.0	81.5	78.5	60.0
	Ferm. and bake loss %	13.6	13.9	14.2	13.6	13.2	12.2	12.9	12.3	13.7

TABLE II

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 2.5% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	670	780	725	595	648	665	565	612	640
	Texture	89.0	91.5	85.5	59.5	77.0	90.5	55.0	71.0	78.0
	Ferm. and bake loss %	13.4	15.9	14.4	12.8	13.1	14.5	12.2	12.6	12.1
2 hr.	Volume cc	842	860	820	748	800	778	692	790	732
	Texture	87.0	90.0	89.0	72.5	92.0	80.0	81.5	88.0	88.0
	Ferm. and bake loss %	15.7	15.4	16.0	15.4	16.1	15.5	14.5	15.4	14.2
3 hr.	Volume cc	745	736	722	772	730	710	738	738	705
	Texture	81.0	84.0	82.5	78.0	85.0	71.5	85.5	86.5	82.0
	Ferm. and bake loss %	15.9	15.9	15.5	16.5	15.8	15.4	15.1	16.0	15.7

100 g flour than nonsoy bread. Higher quantities of soy flour require additional increments of bromate. Loaf volume is also increased with shorter fermentation times, if yeast and bromate are increased to the optimum. With optimum yeast, bromate, and fermentation time,

TABLE III

EFFECT OF VARIATIONS OF SOY FLOUR, BROMATE, AND FERMENTATION WITH 4% YEAST ON BAKING PROPERTIES

Fermentation time	Baking property	No soy flour			3% soy flour			6% soy flour		
		Mg bromate			Mg bromate			Mg bromate		
		3	5	7	3	5	7	3	5	7
1 hr.	Volume cc	822	892	892	748	815	892	638	830	825
	Texture	94.5	91.0	68.5	82.0	94.0	97.0	78.5	87.5	92.0
	Ferm. and bake loss %	16.2	16.8	17.9	14.3	15.7	16.7	13.5	15.4	16.2
2 hr.	Volume cc	840	912	822	872	845	842	845	822	838
	Texture	94.0	89.5	92.5	86.5	95.0	93.5	89.5	81.0	88.5
	Ferm. and bake loss %	17.2	17.2	16.9	16.2	16.7	16.3	16.6	16.4	16.9
3 hr.	Volume cc	765	708	665	778	725	742	782	752	675
	Texture	74.5	66.5	87.0	85.5	84.0	89.0	90.0	77.5	86.0
	Ferm. and bake loss %	19.0	16.3	16.4	17.0	16.8	17.1	16.3	17.4	15.6

bread containing up to 6% of soy flour was approximately equal in volume to the control (nonsoy) bread.

Effect of Soy Flour on Crumb Texture. For optimum texture, extra bromate and shorter fermentation are required where soy flour is used. As is usually the case, the conditions which produce optimum loaf volume are similar to those which give the best texture.

Effect of Soy Flour on Fermentation and Baking Loss. There was a significant lowering of fermentation and baking loss in doughs containing soy flour. This is best shown in Table IV, where the mean values of all of the data are given.

The data show that by relatively minor modifications of fermentation time and bromate level, bread containing 3% of soy flour can be made which compares closely to nonsoy bread. With greater changes in fermentation and bromate, bread of good volume, grain, and texture can be made with 6% added soy flour.

Color of Crumb was not scored individually in these studies because it was early found that the following conditions persisted throughout the whole of the work:

With 3% soy flour and optimum conditions the color of crumb was practically as good as the control. With 6%, the color of crumb was darker, although under optimum conditions even 6% soy flour makes a loaf of bread which would probably be considered quite satisfactory for white bread by the consuming public.

Statistical Studies

The mean values for the effects of the various factors are shown in Table IV. These figures were obtained by averaging all of the results for each factor.

TABLE IV

MEAN VALUES OF MAIN EFFECTS

(All doughs scaled 170 grams immediately after mixing)

Main effect	Loaf volume	Texture score	Grain score	Loaf weight	Fermentation and baking loss
	cc			*g*	%
Yeast (%)					
1.0	503	65	1.84	150.4	11.5
2.5	659	71	2.00	146.8	13.7
4.0	726	74	1.89	145.1	14.7
Soy flour (%)					
.0	659	73	2.00	146.8	13.7
1.5	652	72	1.99	147.2	13.4
3.0	643	71	2.05	147.3	13.4
4.5	626	70	2.02	147.5	13.3
6.0	610	68	2.04	147.8	13.1
7.5	587	66	1.95	148.1	12.9
Bromate (mg)					
1	586	69	2.00	148.7	12.5
3	623	70	2.13	147.5	13.3
5	645	72	2.02	147.2	13.4
7	643	71	1.80	147.2	13.4
9	640	70	1.66	147.4	13.3
11	640	70	1.61	147.4	13.3
Fermentation time (hr.)					
.0	440	60	1.93	151.2	11.1
0.5	544	65	2.00	149.3	12.2
1.0	639	70	2.08	147.9	13.0
1.5	702	72	2.04	146.7	13.7
2.0	707	75	2.04	146.2	14.0
2.5	694	72	2.01	145.5	14.4
3.0	680	72	2.00	144.9	14.8

Figure 3 shows graphically the effect of the various interactions of soy flour, bromate, fermentation time, and yeast on volume and texture.

Table V shows the results of variance analysis for loaf volume data, and the covariance analyses for texture and loss during fermentation and baking. It was found that all of the factors studied had a significant effect on the loaf volume. All of the first order interactions were significant with respect to volume. The data also indicated a significant interrelationship for all four of the factors studied in regard to volume.

After the influence of loaf volume on texture was removed by covariance analysis it was found that none of the factors studied in-

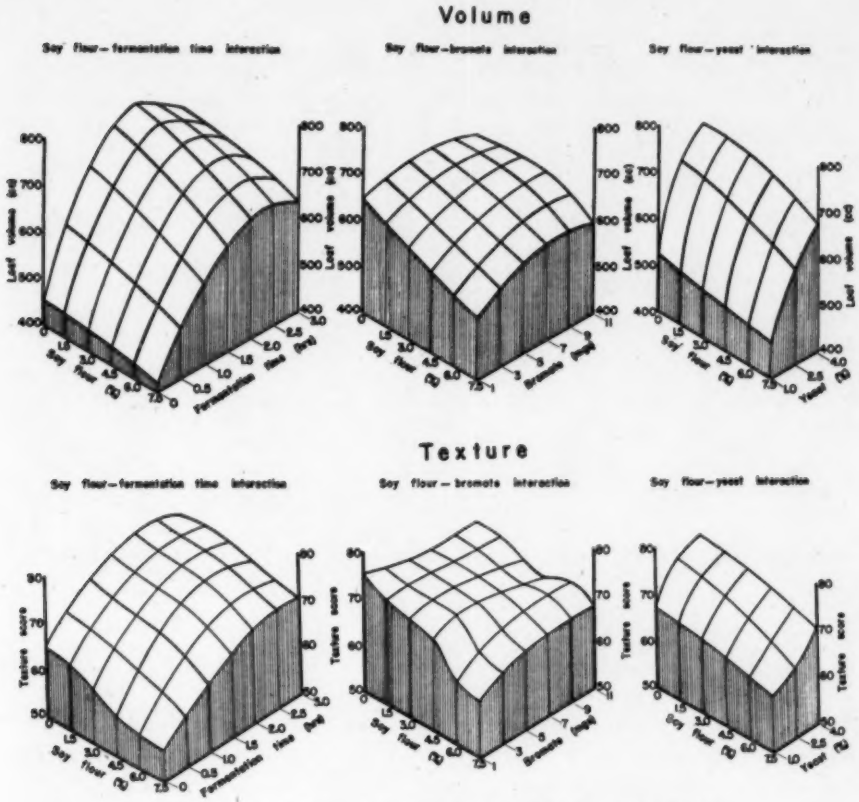

Fig. 3. Volume and texture interactions of formula procedure study.

dependently influenced the texture. However, the first order interactions indicated a significant interrelationship with respect to their influence on texture.

After the influence of loaf volume on the fermentation and baking losses was removed by covariance analysis it was found that percentage of soy flour and fermentation time were of significant influence. The first order interactions were all significant, with the exception of the soy flour-fermentation time interaction.

Summary

With 3% to 6% added soy flour in straight doughs, it was necessary to increase potassium bromate and decrease fermentation in order to produce bread of optimum volume, grain, and texture. For optimum results with 1.5% soy flour 2 mg extra bromate was approximately the best amount. Larger percentages of soy flour required increasing amounts of bromate. Even with as much as 6% soy flour, proper balancing of the factors of yeast, bromate, and fermentation produced

TABLE V

VARIANCE ANALYSES OF THE DATA FOR VOLUME, TEXTURE, AND FERMENTATION AND BAKING LOSS

Source of variation	Volume (variance)		Texture (covariance)		Fermentation and baking loss (covariance)	
	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square
Yeast	2	6,560,632**	2	17	2	145.0
Bromate	5	130,092**	5	260	5	7.8
Soy flour	5	191,861**	5	56	5	20.6*
Fermentation time	6	2,211,680**	6	563	6	187.0*
Interactions						
Yeast × bromate	10	9,095**	10	573**	10	5.0**
Yeast × soy flour	10	11,485**	10	313**	10	5.7**
Yeast × Ferm. time	12	161,399**	12	650**	12	64.2**
Bromate × soy flour	25	6,907**	25	210**	25	5.4**
Bromate × Ferm. time	30	25,224**	30	243**	30	3.9**
Soy flour × Ferm. time	30	8,550**	30	119*	30	1.7
Higher interactions	620	2,763**	1,375	80	620	2.4
Duplicate error	756	710			755	2.1

* Significant at 5% point.
** Significant at 1% point.

bread equal to the control (nonsoy flour) bread except for crumb color. Relatively small changes in these factors were required to produce standard white bread when 3% soy flour was used.

Soy flour reduced the fermentation and baking loss, in proportion to the amount added.

Acknowledgment

Acknowledgment is made to the Soy Food Research Council of the Soy Flour Association, Chicago, for its cooperation and financial assistance in carrying out this project. The assistance of Ralph M. Bohn and Larry Trempel in the preparation of this article is gratefully acknowledged.

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BOOK REVIEWS

Methods of Analysis of the American Society of Brewing Chemists. Fourth Revised Edition. Prepared by Technical Committee and Editorial Board, Louis Ehrenfeld, Editor, 1944. Copies can be obtained from Mrs. Hilda Holmes, Executive Secretary, Route No. 4, Sawyer, Door County, Wis., at the following prices for non-members: Methods $3.50; Extract Tables $2.50; Beer and Wort Tables $2.50.

The present edition in a paper-covered, loose leaf binding represents an extensive expansion and revision of the previous edition. In addition to revised and enlarged sections on Malt, Cereal Adjuncts, Brewing Sugars and Syrups, and Beer, new sections are included on Hops, Wort, Yeast, and Spent Grains.

In the first section on malt, the major changes are an alternative method for bushel weight, employing a 250 ml graduated cylinder and a special funnel, and the ferricyanide modification for the determination of diastatic power. Methods for moisture, extract, and color in caramel malt and for moisture and color in black malt are included for the first time.

The section on brewing sugars and syrups includes for the first time methods for the determination of hydrogen ion concentration (pH) and total reducing sugars. A rapid fermentation method (4–5 hours) is given for fermentable extracts. The Fehling's solution procedure only is given for the determination of diastatic power of malt syrups. The ferricyanide modification should be as suitable for this material as for malt if a blank determination is carried out on each sample and the necessary corrections made in the calculation.

To the section on beer has been added methods for the determination of dextrins and calibration of hydrometers. These additions complete a very comprehensive collection of methods for the analysis of beer.

The new section on hops includes methods for sampling, physical examination, moisture, and resins. Ten methods which are in common use for the analysis of wort are combined in a new section. A method for total solids in liquid and pressed yeast is given in the section on yeast. To complete the book a section on spent grains gives procedures for moisture, available extract, and soluble extract and one is referred to the A.O.A.C. for feed analysis methods.

The third edition of the tables for Extract Determination in Malt and Cereals represents an enlargement of the previous edition to include higher specific gravities and higher moisture contents. These changes make the tables more suitable for determination of yield of extract in cereals.

The tables relating to wort, beer, and brewing sugars and syrups are a corrected reprint of the earlier edition except for Table 4, "Reducing Sugar Values by the Munson and Walker Method," which has been greatly expanded. This booklet contains tables for Extract in Wort and Beer, Alcohol, Correction Factors for Original Extract, Reducing Substances (Munson-Walker), Reducing Substances (Lane-Eynon), and Baume Degrees as related to extract content.

The book of methods and accompanying tables is a very complete and useful publication especially for brewery, brewing, and malting laboratories.

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Physical Methods of Organic Chemistry. I. Edited by Arnold Weissberger, Eastman Kodak Company. Interscience Publishers, Inc., New York, N. Y. 1945. 736 pp., 15 × 23 cm. Price $8.50.

The purpose of this book, as stated in the preface, is to provide an adequate description of important physical methods that are of proven usefulness to chemists. The object of the editor and the authors, who are experts in the method each describes, is to present the techniques in sufficient detail together with enough of the theoretical background so that those who are interested in their use may be saved the tedious and oftentimes difficult job of assembling the "know how" from periodicals and specialized books.

The methods discussed in this volume, 16 in number, together with the authors and the number of pages devoted to each are as follows: I. Determination of Melting and Freezing Points—E. L. Skau and H. Wakeham (46 pp.). II. Determination of Boiling and Condensation Temperatures—W. Swietoslawski (22 pp.). III. Determination of Density—N. Bauer (38 pp.). IV. Determination of Solubility—R. D. Vold and M. J. Vold (28 pp.). V. Determination of Viscosity—H. Mark (14 pp.). VI. Determination of Surface and Interfacial Tension—W. D. Harkins (53 pp.). Including a discussion of the Parachor—G. W. Thomson (9 pp.). VII. Determination of Properties of Monolayers and Duplex Films—W. D. Harkins (42 pp.). VIII. Determination of Osmotic Pressure—R. H. Wagner (24 pp.). IX. Determination of Diffusivity—A. L. Geddes (34 pp.). X. Calorimetry—J. M. Sturtevant (124 pp.). XI. Microscopy—E. E. Jelley (96 pp.). XII. Determination of Crystal Form—M. A. Peacock (30 pp.). XIII. Crystallochemical Analysis—J. D. H. Donnay (24 pp.). XIV. X-Ray Diffraction—I. Fankuchen (36 pp.). XV. Electron Diffraction—L. A. Brockway (32 pp.). XVI. Refractometry—N. Bauer and K. Fajans (84 pp.).

Of particular advantage to the uninitiated is the aim of some of the authors to evaluate critically the various modifications of the method under discussion, pointing out the specific advantages or shortcomings of each modification and the conditions under which each may be expected to yield the most dependable results. Proper interpretation of results is stressed and illustrated by examples in many cases. Apparatus to be employed is described by diagrams and detailed discussion. Numerous literature citations appear along with the text and references of a general character are included at the end of each chapter.

The usefulness of this volume is by no means restricted to organic chemists or even to chemists actively engaged in research. Advanced students will find the critical descriptions of practical methods helpful to their understanding of the theoretical aspects involved.

The editor and publishers have done a commendable job. There are few errors.

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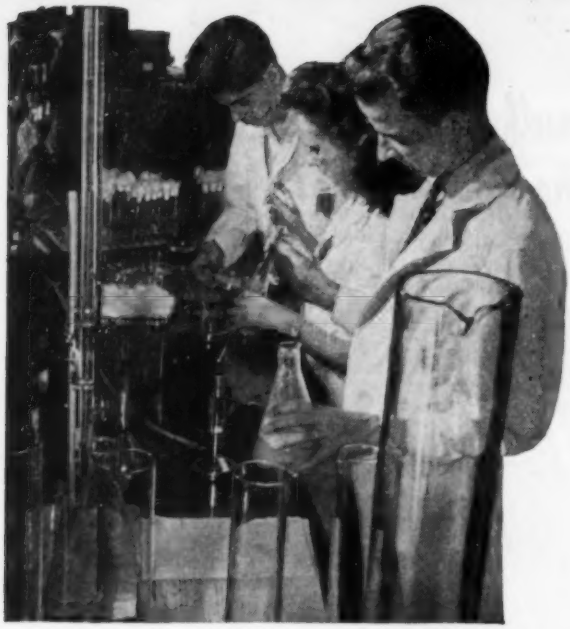

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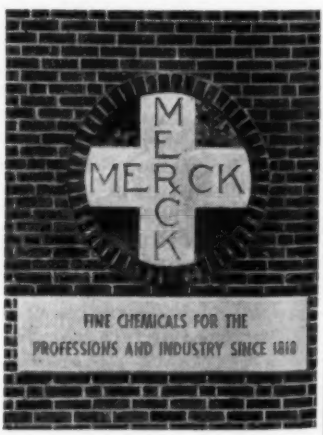
In a number of instances, the pure vitamins may be considered to be products of Merck research. Several were originally synthesized in the Merck Research Laboratories, and others have been synthesized by Merck chemists and collaborators in associated laboratories.

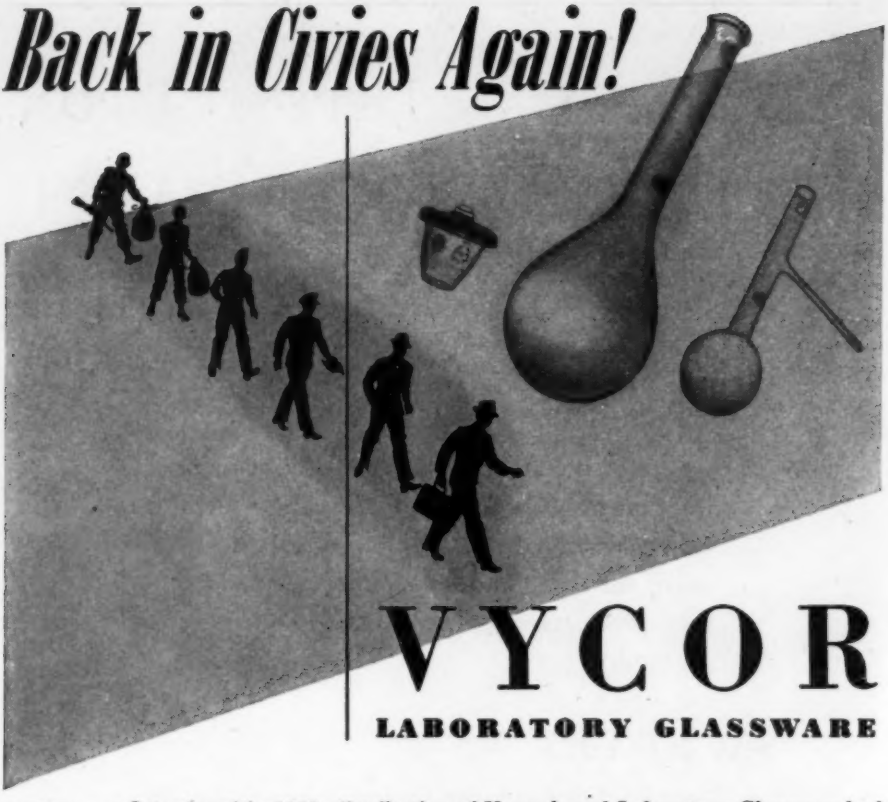
Because most of the known vitamins have now been made available in pure form, effective therapy of specific vitamin deficiencies can be conducted on a rational and controlled basis, under the direction of the physician.

Merck & Co., Inc. now manufactures all the vitamins commercially available in pure form, with the exception of vitamins A and D.



MERCK & CO., Inc. *Manufacturing Chemists* **RAHWAY, N. J.**

Back in Civies Again!



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LABORATORY GLASSWARE

• Introduced in 1939, distribution of Vycor brand Laboratory Glassware had barely started when World War II began and most of the ware was drafted.

Now, however, "Vycor" is back in civies again and is being stocked by your laboratory supply dealer.

It is fabricated from Vycor brand 96% silica glass by a unique, patented method whereby most of the constituents other than silica are leached out. It has a very low co-efficient of expansion (approximately .0000008 per °C, from 0° to 300° C), an extremely high softening point and exceptional chemical stability. Consequently, it is ideal for many types of important laboratory work. Reactions can be carried out at higher temperatures with greater speed and accuracy. In fact where use of fused silica was previously indicated, "Vycor" Glassware will ordinarily serve equally well at much lower cost.

A sufficient variety of shapes—beakers, crucibles, evaporating dishes, tubes, etc.—is now available to provide a fairly complete coverage of most upper temperature requirements. Consult your laboratory supply dealer.

Vycor is a registered trade-mark and indicates manufacture by

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VYCOR FABRICATED FROM VYCOR 96% SILICA GLASS
LABORATORY GLASSWARE

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Mrs. Jones is making a decision . . . a momentous one for the makers of the two products she holds in her hands.

Both packages are practically identical in content . . . comparable in quality and price. Yet a simple statement on one of them has caught her eye and influenced her decision. It is the statement that *this* particular product has been fortified with essential minerals.

According to nutrition authorities, two out of every three persons are not getting enough minerals in their foods. Here is a fact that is being hammered home to the public day after day.

Other things being equal, isn't it only natural that Mrs. Jones will decide in favor of the product that provides the extra nourishment authorities agree is so essential to good health?

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Food Minerals

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Dicalcium Phosphate
Tricalcium Phosphate
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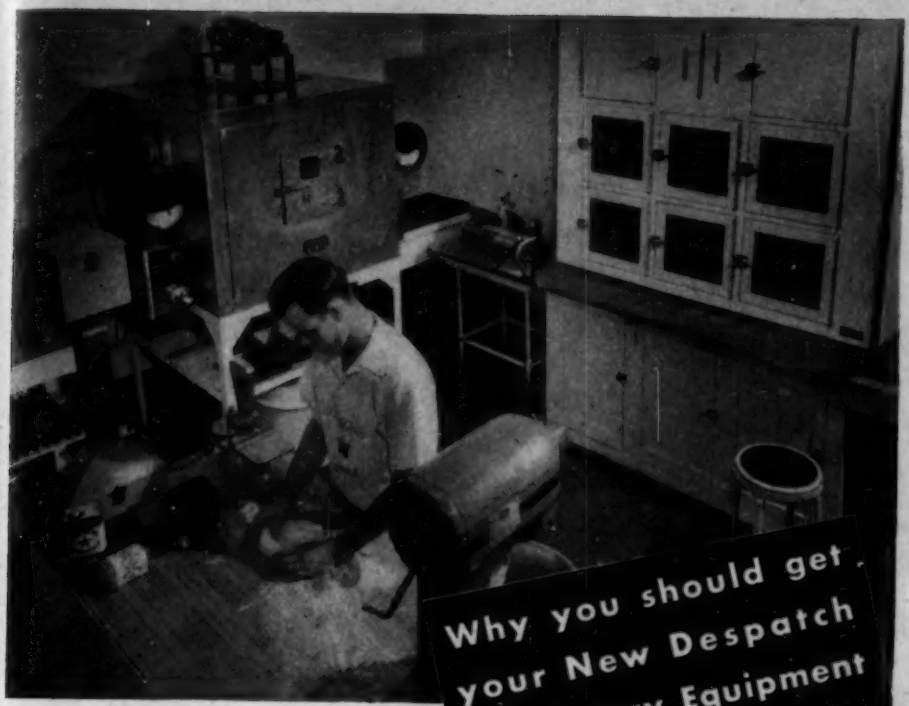

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